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AN ANALYSIS OF THE FACTORS INFLUENCING GROWTH AND DEVELOPMENT OF THE MAMMALIAN REPRODUCTIVE TRACT[1]

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I. Introduction

A comprehensive analysis of the factors which control the development of the reproductive system of mammals should treat at least four broad aspects of the problem. These are (1) the availability to the developing organism of endogenous and exogenous hormones; (2) the ability of the various parts of the developing system to react to such hormones; (3) the relation of the degree of sensitivity of the various end-organs to age, sex, genetic strain, endocrine factors, and environmental influences; and (4) the amount of growth and development which is not dependent upon hormone influence.

The developing organism grows prenatally in a complicated hormone environment. From the time of implantation to the end of pregnancy, hormones from the placenta may be available to the fetus, and at certain periods the placenta is permeable to maternal hormones. These hormones vary quantitatively and qualitatively during pregnancy, but high titers of gonadotrophic and female sex hormone are present in the mother and normally reach the fetus, at least in some species, in effective amounts.

There is convincing evidence that fetal hypophyses, gonads, adrenals, and thyroids of many species assume their hormone-producing function in the course of their prenatal development and that some, if not all, of these hormones circulate in the fetal blood stream.

At birth the newborn mammal is dependent upon its mother's milk, which has been shown in several species to contain estrogenic and gonadotrophic hormones for a period of time. This adds a source of exogenous hormones, and suckling young have been found to be affected by such maternal hormones. In postnatal development the various endocrine glands reach (or have already reached at birth) their functional state; the complicated interrelationships of gonads, hypophyses, thyroids, and adrenals are established; and hormones from the gonads assume the major role in the development of the accessory organs, such as the uterus, the prostate, the seminal vesicles, etc.

Sensitivity to hormones is present during fetal life in the mammalian reproductive tract. This has been proved by extensive experimental studies in the rat, mouse, guinea pig, and cat and by observations on the normal fetus and newborn human, monkey, horse, pig, and guinea pig.

It has been established that postnatally the gonads and accessory organs can be stimulated by hormones from many sources. The rat and mouse furnish striking examples of this great reactivity. The ovary and testis respond to gonadotrophic hormone; but, in addition, the ovary is stimulated directly by estrogen, and the testis by androgen. The accessory reproductive organs of the male rat and mouse react to androgens from the testis and the adrenal. In the rat, ovarian androgen is also effective in stimulating the male and female prostate glands and the seminal vesicle. The uterus of the rat and mouse respond to estrogen from the ovary and adrenal, and also to androgen. The male and female rat mammary

glands are responsive to estrogens and androgens. It is a striking fact that each of these organs can react to hormones from two or more sources, but the significance of this reactivity in determining the course of development is not yet clear.

Although the capacity to respond to various hormones may be called an almost universal character of the gonads and accessory reproductive organs, the degree of responsiveness is influenced by a number of factors. It is dependent on age, sex, and strain of the animal; on the normal function of other endocrine glands; and on diet, vitamins, light, etc. The means by which reactivity is increased or decreased and the possible hormone interrelationships are little understood.

Growth and development of parts of the reproductive tract can proceed in animals deprived of the endocrine glands which normally provide the stimulation for the major portion of such growth and development. The gonads of young hypophysectomized rats and mice continue growth and differentiation for a period of time following the operation, but their ultimate development is very limited. The prostate glands of young rats, mice, and opossums continue development after gonadectomy. However, in rats and mice in which adrenalectomy and gonadectomy have been performed, it has been shown that the adrenal gland is a source of stimulation after the gonads are removed. In rats there is only very limited growth of the seminal vesicle in the absence of the testes, but a part of that growth is dependent on hormones from the mother's milk. The seminal vesicle of the castrated mouse apparently develops because of adrenal hormones. The uteri of young rats, mice, and opossums continue development in the absence of ovaries, but in the rat and mouse the adrenal cortex provides estrogenic hormone after gonadectomy.

The ability of the accessory organs of reproduction to react to hormones from several sources, the availability of a hormone which can completely or partially substitute for the gonad-produced hormone, and even the possibility that hormones present in the mother's milk may for a time affect the developing young greatly complicate any analysis of the amount of organ growth that is definitely not hormone-conditioned.

From this brief discussion it can be seen that the development of the mammalian reproductive tract presents many interesting problems. It would be impossible in this paper to treat them all adequately. In the past few years excellent reviews have dealt with such subjects as the relation of hormones to early growth and differentiation of the reproductive tract (Moore, 1941*a*, 1944; Burns, 1942*a*, *b*, and 1945; Greene, 1942; Raynaud, 1942), the endocrinology of gestation (Courrier, 1945), adrenal-gonad relationship (Parkes, 1945).

In this study some of the problems will not be discussed, and others will receive only brief treatment; but, as far as possible, an attempt will be made to integrate the factors which may be normally influencing the prenatal and postnatal development of the gonads and accessory reproductive organs. The problems of prenatal development will be discussed in relation to a number of species of animals, but the analysis of postnatal growth and development will be limited specifically to the rat.

II. Prenatal Development

A. Availability of hormones to the fetus

The developing fetus has three possible sources of hormones which might influence the growth and development of

the reproductive tract: (1) maternal, (2) placental, and (3) fetal.

1. MATERNAL AND PLACENTAL HORMONES

It has been known since the early work of Zondek and Aschheim that estrogen and gonadotrophin are present in the urine and blood of pregnant females. The high titer of gonadotrophic hormone in the urine of pregnant women provides the basis for the well-known Zondek-Aschheim pregnancy test. The development of that test for gonadotrophin and the Allen-Doisy method for the determination of estrogen stimulated many studies on the occurrence of these hormones in the body fluids, tissues, and organs during pregnancy. Because of the importance of the Zondek-Aschheim test for diagnosing pregnancy in the human and the possible significance of high titers of estrogen in pathological conditions, much of the early research was done on humans. However, such studies were extended to other species, and a large body of information has accumulated. Much of this research, with the addition of more recent work on progestin, is summarized in Table 1.

The data on the occurrence of hormones in the pregnant female have been limited to the presence of hormones in blood, urine, and colostrum, which are not, of course, the only hormone-containing parts of the body. Estrogen is found in the pregnant mare in ovary, endometrium, kidney, urine, blood, and feces (Catchpole and Cole, 1934). Heim (1932) listed the finding of gonadotrophin in pregnant women in the anterior hypophysis, epiphysis, corpus luteum, salivary glands, thyroid, thymus, pancreas, gall, adrenal, stomach glands, Bartholin's glands, urine, blood, and feces.

In Table 1 it can be seen that estrogen has been extracted from the urine and blood of pregnant females of all species examined, from the human to the rat. The amounts present vary widely according to species and at different periods of pregnancy. In the cow and human, estrogen content in the urine rises steadily to parturition and then drops, while in the mare estrogen appears in the urine in large amounts by the 74th day (Zondek, cited by Cole and Saunders, 1935), reaches a peak between the 200th and 275th day, and then falls gradually to term at about 336 days (Cole and Saunders, 1935). The estrogen titer is relatively low in the blood of pregnant mares from 150 days to term, but the amount excreted in the urine is very high and may be ten times that found in the urine of pregnant women. In spite of such species differences, circulation of estrogen in the blood and estrogen excretion in the urine are of general occurrence in pregnancy. Dorfman and Van Wagenen (1941) provided direct evidence from their study of the macaque that estrogens may be produced in the ovary, placenta, and adrenal cortex during pregnancy and are excreted in increased amounts.

Gonadotrophic hormone is not such a constant finding in the urine and blood of different species during pregnancy (see Table 1). In general, gonadotrophin has been found in the urine in species in which its presence has been reported in the blood (the cow is an exception); but in the mare, for example, there are only small quantities in the urine and extremely large amounts in the blood, while in women the high titer in the urine is diagnostic of pregnancy. Gonadotrophin can be demonstrated in the urine of pregnant women at about the 40th day, rises rapidly to a peak between 50 and 60 days, and falls to a low level by 80 days, which is maintained to term (Browne and Venning, 1936). The dimi-

TABLE 1

OCCURRENCE OF ESTROGEN, PROGESTIN, ANDROGEN, AND GONADOTROPHIN IN BODY FLUIDS OF THE PREGNANT FEMALE AND THE FETUS, AND IN FETAL ORGANS AND PLACENTAE

Pregnant Female			Membranes and Fluids		Fetus					
Urine	Blood	Colostrum or Milk	Placenta or Chorion	Amniotic Fluid	Fetus	Blood	Testis	Ovary	Liver	Kidney
Estrogen										
Human +	Human +	Human +	Human +	Human +	Human +	Human +	.....	.....	.....	.....
Chimpanzee +	.....	.....	Chimpanzee +	.....	.....	.....	.....	.....	.....	.....
Macaque +	Macaque +	.....	Macaque +	.....	.....	.....	.....	.....	.....	.....
Horse +	Horse +	.....	Horse +	.....	.....	Horse +	Horse +	Horse +	Horse +	Horse +
Cow +	Cow +	Cow +	Cow +	Cow +	.....	Cow +	.....	Cow +	.....	.....
Goat +	Goat +	Goat +	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	Sheep +	Sheep +	.....	.....	.....	.....	.....	.....	.....
Pig +	Pig +	.....	Pig −+	Pig −	Pig −+ (♂ and ♀)	.....	.....	.....	Pig + (♂ and ♀)	.....
.....	.....	.....	Dog −	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	Cat −+	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	Beaver +	.....	.....	.....	.....	.....	.....	.....
.....	Rabbit +	.....	Rabbit +	Rabbit +	Rabbit +	.....	.....	.....	.....	.....
.....	.....	.....	Guinea pig −+	.....	.....	.....	.....	.....	.....	.....
Rat +	Rat +	.....	Rat −?	.....	.....	.....	.....	.....	.....	.....
Gonadotrophin										
Human +	Human +	Human +	Human +	Human +	Human +	Human +	.....	.....	.....	.....
Chimpanzee +	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Orangutan +	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Macaque −+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Horse +	Horse +	.....	Horse +	Horse −	Horse − (a few +)	.....	.....	.....	Horse − (a few +)	.....
Cow −+	Cow −	.....	Cow −	.....	.....	.....	.....	.....	.....	.....
Goat −	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	Sheep −	.....	Sheep −	.....	.....	.....	.....	.....	.....	.....
Pig −	Pig −	.....	Pig −	.....	.....	.....	.....	.....	.....	.....

TABLE 1—*Continued*

Pregnant Female			Membranes and Fluids		Fetus					
Urine	Blood	Colostrum or Milk	Placenta or Chorion	Amniotic Fluid	Fetus	Blood	Testis	Ovary	Liver	Kidney
Gonadotrophin—*Continued*										
Dog −										
Cat −										
Rabbit −	Rabbit −									
	Guinea pig −									
Rat −	Rat −									
	Mouse −									
Elephant −										
Giraffe +										
Progestin										
Human* +	Human +		Human +							
Horse* +			Horse +				Horse −	Horse −		
Cow* +			Cow +							
			Goat +							
Cat* −										
Rabbit* −										
Androgen										
Human +										
Macaque +							Horse +	Horse +		
						Bull +	Bull +			

* Pregnanediol.

NOTES FOR TABLE 1

The plus sign (+) represents one or more positive findings.

The minus sign (−) represents one or more negative findings.

Some of the data are from literature not cited in the context; e.g., E. Allen, 1932; W. Allen, 1939; Corner, 1938; Courrier, 1945; D'Amour, Funk, and Glendenning, 1936; Doisy, 1941; Engle, 1932 and 1939; Frank, 1929; Newton, 1939.

nution at 60 days coincides with a rising titer of estrogen.

Gonadotrophin is found in the blood of pregnant mares by the 45th day (fetal C.R. length of 2.5 cm.), and the amount increases with extreme rapidity, reaching a maximum at 70 days. This level is maintained to about 100 days, when it falls rapidly, and gonadotrophin disappears from the blood by about 175 days. The decline in gonadotrophin coincides with the increase in urinary estrogen. Negative findings for gonadotrophin in both blood and urine have been reported for a number of species, including the goat, sheep, pig, dog, cat, rabbit, guinea pig, rat, mouse, and elephant.

Assay methods for progestin have been more tedious than those for estrogen and gonadotrophin, and not so many species of animals have been examined for its presence. Progestin has been found in the blood of pregnant women and, in the form of pregnanediol, it occurs in the urine of the pregnant woman, horse, and cow (Courrier, 1945). Cat and rabbit urine has been found negative. In the pregnant woman the studies of Browne, Henry, and Venning (1937) have shown that the amount of pregnanediol in the urine increases during pregnancy from the 3d month (4–10 mg. daily) to the 8th month (73–80 mg. daily) and practically disappears by 24 hours after delivery.

Androgens have been reported in the urine of pregnant women by Womack and Koch (1930) and by Marker and Lawson (1938); but Hain (1939) found a lowered excretion during pregnancy as compared with the amount in the urine of nonpregnant women. In the monkey, however, Dorfman and Van Wagenen (1941) found increased amounts of androgens in the urine during pregnancy, and they concluded from their studies that the greatest part was derived from the adrenal cortex. The prostate gland of the pregnant rat shows evidence of stimulation, indicating an increased amount of androgen in pregnancy (Burrill and Greene, 1942).

The importance, if any, of all these hormones in influencing fetal development depends upon whether they reach the fetus and circulate in the blood stream. The placenta has been shown to be permeable to many substances, such as hormones, drugs, etc., injected into pregnant females; but permeability of the placenta need not be essential, since estrogen, gonadotrophin, and progestin are probably secreted by the placenta itself. Newton (1939, p. 729) states: "The placenta is now thought to be the source of the extra-ovarian estrin produced in women and mares and probably other animals during pregnancy, and also of the 'gonadotrophic' hormone of pregnancy where this occurs." In discussing progestin in the human placenta, he concludes (p. 732): "The quantity obtained is small but in view of the extra-ovarian source during pregnancy it gives the placenta an important claim to be considered as the source." Courrier (1945) reaches the same conclusion.

In Table 1 positive findings of estrogen are listed for all placentae except those of the dog, cat, and rat. Courrier suggests that in negative cases, such as the dog and cat, the extraction methods may not have been so effective as those developed later, and the findings of estrogen in the cat placenta by other workers seem to confirm this. For the rat, Allen and Doisy (1927) state that the tests were made on small amounts of material and that larger quantities should be tried before a definite statement can be made. It seems probable that estrogen is normally present in all placentae. The hormone is found in both the maternal and the embryonic parts of the placenta.

In contrast to the wide occurrence of

estrogen, gonadotrophin has been found in placental extracts of only the human and the horse, but not many species have been tested. In the horse (Catchpole and Lyons, 1934), gonadotrophin appeared in the chorion when the fetus had a crown-rump length of only 1.8 cm., and the hormone was still found in the chorion for a short time after it had disappeared from the blood. Catchpole and Lyons postulate a chorionic origin of this gonadotrophin.

Although no reports of extraction of gonadotrophin from rat placentae have been found in the literature, Astwood and Greep (1938) demonstrated a corpus luteum–stimulating substance in rat placentae and concluded that hormone from the fetal placenta is responsible for maintaining functional corpora lutea in the latter half of pregnancy in the rat. The active material was stated to be unlike any known gonadotrophic substance. A trophic influence of the mouse placenta on the ovary has been described by Newton and collaborators.

Progestin has been found in extracts of placentae of the human, horse, cow, and goat. A number of workers have produced indirect evidence that the rat placenta secretes progestin (see Newton, 1939, p. 733).

The findings on hormones in placental tissues are in agreement with those for hormones in urine and blood in that, when the hormone is present in urine and blood, it is found also in the placenta.

The amniotic fluid of a number of species contains estrogen, and large amounts of the hormone are present in both the amniotic and the allantoic fluid of the cow (Morrell, Powers, and Varley, 1930). Human amniotic fluid contains gonadotrophic hormone.

2. HORMONE OCCURRENCE IN THE BLOOD AND ORGANS OF THE FETUS

Evidence of the presence of hormones in the blood and organs of the fetus may support the possibility that maternal or placental hormones are reaching the fetus. It is necessary to consider, also, that fetal organs may be contributing hormones (see below, p. 221). However, there is evidence that hormones have been found in chorionic vesicles before the period when fetal organs would be expected to be functioning. Estrogen was found in intact human chorionic vesicles containing normal 12.5-mm. and 20-mm. embryos, the latter half of the second month of pregnancy (Allen and Doisy, 1927). The sex of the gonad cannot be determined at 12 mm., and the Müllerian ducts are just forming at this time (Arey, 1942, p. 148). Gonadotrophin has been reported in the human chorionic vesicle when 2 or 3 weeks old, and the cells of the chorion have been suggested as the hormone source (Maroudis, 1933).

As shown in Table 1, estrogen has been found in the human, pig (♂ and ♀), and rabbit fetus and in fetal human, horse, and cow blood. The following organs yielded estrogen: human fetal liver and kidney; fetal horse testes, ovaries, liver, and kidney; fetal cow ovary; and pig liver (♂ and ♀).

There has been little research on the gonadotrophic content of the fetus, but the hormone has been found in extracts of the human fetus and in cord blood. A few positive cases were reported for extracts of the horse fetus and fetal liver. Extracts of horse testis and ovary were negative for progestin.

Androgen has been demonstrated in fetal bull blood and testes and in fetal horse testes and ovaries.

While many of these hormones are probably derived from the placenta or from the mother, there is evidence that

fetal organs may be contributing hormones.

3. EVIDENCE FOR THE FUNCTION OF FETAL ORGANS

There is abundant evidence that many fetal organs secrete their definitive hormones before birth. The fetal hypophysis produces growth hormone, gonadotrophin, thyrotrophin, and lactogen (see Reece and Turner, 1937); the thyroid, its hormone; the pancreas, insulin; and the adrenal, epinephrin. Windle (1940) has reviewed much of the research on the fetal endocrine glands and the evidences of their function. It is recognized that the demonstration of hormone in a gland does not necessarily prove that that hormone is released to circulate in the blood stream. However, an increasing amount of evidence points to fetal function of the endocrine organs and indicates the establishment of some of their hormone interrelationships before birth.

The gonads would be of most importance in influencing the development of the fetal reproductive tract. As reported above, male hormone has been extracted from fetal bull testes (Womack and Koch, 1932; and Atsumi, 1939*a*). Demonstration that the hormone was released and circulating in the blood stream was given by Atsumi, who claimed that androgen was found in extracts of fetal bull blood from the umbilical arteries and not in blood from the umbilical vein or maternal carotid. Thus he claimed that it could not have come from the placenta or the mother and must have been produced in the fetus itself. He also reported (1939*b*) that estrogen was extracted from fetal cow ovaries and that the hormone was found in the blood from the umbilical artery and not in the umbilical vein or carotid of the mother in the earlier part of pregnancy. Later in pregnancy, estrogen was found in the umbilical vein and in the mother's carotid. These experiments indicate that in this species the fetal testis and ovary are functioning and releasing their hormones.

Both androgen and estrogen have been found in extracts of fetal horse testes (Catchpole and Cole, 1934). The finding of androgen indicates hormone secretion in the fetal testis. The estrogen may have come from the placenta, but, since estrogen has been extracted from stallion testes (see Doisy, 1941, p. 5), its presence in fetal testes may also indicate testis function. The estrogen that was found in fetal horse ovaries may have been produced by the ovaries, or it may have come from the placenta.

Recent research employing the technique of embryonic castration has yielded interesting results. Wells (1946) castrated male rat embryos between 19 and 20 days post coitum (or later) and killed them at 21 days and 16 hours, just before expected delivery. In spite of the short period of castration, he was able to demonstrate a reduction in the volume of the seminal vesicles and bulbo-urethral glands as compared with the normal, indicating that the fetal rat testes were producing effective amounts of hormone.

Jost (1947*a*) performed embryonic castrations on the male rabbit at 21 or 22 days post coitum (he states that somatic sexual differentiation begins at 20 days) and killed them at 28 days post coitum (gestation period 32 days.) His results are striking. Removal of both testes caused Wolffian duct regression, absence of the seminal vesicle, and inhibition of the prostate. These experiments are a clear demonstration of hormone function in the fetal rabbit testis. In further experiments (1947*b*) he gonadectomized littermate males and females at 20 days and 20 hours post coitum and

killed them at about 27 days. The results on the male were even more striking than in the earlier experiments. The results of spaying the female were not so marked, but the Müllerian ducts of spayed females were smaller than those in the normal. Jost states that it is possible that the Müllerian ducts receive stimulation from the ovary in the early stages of their organogenesis. From this there seems to be a possibility that the fetal rabbit ovary may secrete small amounts of estrogen.

No discussion of the function of fetal gonads would be complete without a consideration of the freemartin and the evidence that it offers. Lillie (1917) decribed the freemartin, an intersexual fetal female calf that is co-twin to a male, and suggested that the partial sex reversal of the female was the result of male hormone influence from the normal male co-twin. In support of this contention was the finding of anastomoses of blood vessels in the placenta so that the blood of the male twin could mix with that of the female. Lillie's interpretation of the freemartin condition as stimulation (and inhibition) produced by testis hormone from the male twin provided a basis for a theory of normal sex differentiation, which will be discussed below. For our purposes here, the freemartin offers evidence that the fetal bull produces a substance which influences the female co-twin. The extraction of androgen from fetal bull testes and its presence in the blood stream of the fetal bull (cited above) support the idea that this substance may be male hormone from the fetal testes.

Hughes (1927, 1929) reported that the freemartin condition occurred also in pigs when the membranes of two fetuses of opposite sex were fused.

From experimental evidence and from studies of the freemartin condition, it seems established that fetal rat, rabbit, horse, and bull testes secrete male hormone; that fetal cow, and possibly rabbit, ovaries produce female hormone; and that both hormones are released in the blood stream.

From the data given in Table 1, it is evident that the developing fetus (♂ and ♀) of many species contains estrogen. Gonadotrophin appears in the human and horse fetus, but it may have a wider distribution than has yet been demonstrated. Androgen occurs in high concentrations in fetal bull and horse testes and in bull blood and has been reported in the horse ovary. The presence of these hormones, some of which are apparently secreted in the fetus, can have no significance unless the fetal end-organs are capable of reacting.

B. EVIDENCE FOR REACTIVITY TO NORMAL PRENATAL HORMONE STIMULATION IN THE FETUS AND NEWBORN ANIMAL

It must be emphasized that the stimulating effects of hormones normally present in the fetus can be recognized only by "abnormal" development, that is, by changes in organ size, histology, and function that (1) are typically produced by hormone action in the postnatal animal and (2) undergo regression at a later period or after birth. This means that only striking and maximal effects can be detected.

Table 2 presents evidence that, in species as different as man and guinea pigs, hormones have a prenatal influence on the reproductive tract. Halban (1904) reported that the fetal uterus showed female hormone stimulation by the 8th lunar month, the mammary glands of the female and male by the 7th, and that the prostate gland of the male gave evidence of male hormone stimulation. He postulated that a "pregnancy substance"

TABLE 2

THE PRESENCE OF HORMONES IN BODY FLUIDS OF THE NEWBORN, AND EVIDENCE OF HORMONE STIMULATION IN THE FETUS AND NEWBORN

Hormone Producing Stimulation	Evidence of Stimulation							Presence of Hormones in the Newborn		
	Organs						Behavior			
	Ovary	Uterus	Vagina	Mammary Glands	Testis	Prostate	"Heat Reaction"	Urine	Blood	Colostrum
Estrogen		Human +	Human +	Human +				Human +	Human +	Human +
		Macaque +		(♂ and ♀)						
				Horse +						
		Guinea pig +	Guinea pig +	Guinea pig +			Guinea pig + (♂ and ♀)			
Gonadotrophin	Human +				Human +			Human +	Human +	
	Horse +				Horse +					
	Donkey +				Donkey +					
					Pig +					
Mammotropin				Human + (♂ and ♀)				Human + (♂ and ♀)		
				Horse +						
				Guinea pig +						
Progestin				Human +				Human +		
				Horse +						
				Guinea pig +			Guinea pig + (♂ and ♀)			
Androgen						Human +				

came from the placenta. Halban (1905) found fetal mammary growth and secretion of "witches' milk," which he explained on the basis of hormones circulating in the maternal blood stream. Scammon (1926) studied a series of human uteri and found that the fetal uterus grows at a slow and constant rate until the 7th lunar month and then undergoes rapid linear growth, doubling in length by the 10th lunar month. There is marked reduction in length and weight after birth. He suggested a "substrate of typical fetal growth plus a secondary growth increment, which presumably is due to an extra stimulus furnished by a hormone of placental or possibly ovarian origin." The uteri of fetal and newborn human females are not only large but hyperemic, and uterine bleeding occurs at birth in a certain number of cases. Halban described the histology as similar to that found in menstruating women.

Neumann (1930, cited by Engle, 1932) described uterine and vaginal hyperemia at birth, as well as polycystic ovaries; and other workers have reported the same phenomena with the vaginal reaction appearing at the beginning of the 5th month (see Courrier, 1945, p. 337). Fraenkel and Papanicolaou (1938) found that vaginal fluid of newborn babies contained squamous cells, and sections of the vagina showed that the epithelium was hypertrophic. The vaginal smears began to show regressive changes soon after delivery. They also observed stimulation in the ovarian follicles and mammary gland hypertrophy and secretion. Their conclusions were that estrogenic action was evident in the epithelial hypertrophy, that gonadotrophic influence was apparent in the ovaries, and that "the presence of both estrogenic and the gonadotrophic hormones may explain the secretion of witches' milk." They thought that the hormones came from the mother but that the stimulated ovaries might also be producing estrogen.

Mammary hyperplasia is very common in male and female babies at birth and for a short time afterward, and lactation (colostrum followed by a small amount of milk) occurs frequently. There is no sex difference in the appearance of this phenomenon. Lyons (1937) found mammotropin in the urine of four newborn children. One, a boy, had unusually large amounts in the urine and secreted colostrum for 2 days and milk for the remaining 5 days that he was under observation. The mammotropin presumably came from the mother, as did the estrin necessary to stimulate the glands. Mammotropin is present in the urine of lactating women (Lyons and Page, 1935).

Prostatic hypertrophy, increase in interstitial cells in the fetal human testis, and damage to testis tubules have been described by a number of workers (see Engle, 1932; and Courrier, 1945). Kitahara (1923) found that interstitial cells began to appear in abundance in the human testis by the beginning of the 4th month. Engle (1939, p. 791), in discussing these changes in the testes, states: "It is logical to regard these changes in the tubules as due to the long continued action of the female sex hormones or one of the anterior-pituitary-like factors; while the interstitial hypertrophy is a direct response to the anterior-pituitary-like factors in the placental blood."

R. A. Moore (1936) described differentiation and physiological activity in some of the acini of the human prostate at birth and soon after. In the next 4–6 weeks the hyperplastic acini were found to involute.

All these studies and many more suggest strongly that the human fetus and newborn child have been under prenatal hormone influence, in some cases several months before birth. Estrogen, gonado-

trophin, progestin, and mammotropin have all been effective, and androgen from fetal testes has probably been the stimulating substance in prostate hypertrophy. That such hormones have been circulating in the fetus is proved by the recovery of estrogen and gonadotrophin in the urine and blood of the newborn male and female (Philipp, 1929; Joseph, 1929; Brühl, 1929; Winter, 1932), mammotropin in newborn male and female urine (Lyons, 1937), and progestin in urine of newborn children (Philipp, 1936; Holtz and Wöllpert, 1937, cited by W. Allen, 1939). A placental or maternal origin for some of these hormones is indicated by the fact they they disappear from the blood and urine a few days after birth, as does also the estrogen which appears in the colostrum and milk of newborn children (Joseph, 1929). As can be seen in Table 2, in the human, in all organs except the prostate, when evidence of stimulation was found, the hormones that were capable of producing that stimulation were present in the urine (some also in the blood and colostrum) of the newborn. No reports were found in the literature of research on the androgenic content of the urine of the newborn child.

Evidence of prenatal hormone effects on the fetus are by no means limited to the human. Courrier (1930, 1945) has discussed what he terms "la crise génitale du nouveau-né," which appears as a sort of temporary "puberty" in man and other animals. He has observed in some guinea pigs at birth marked vaginal changes and uterine and mammary gland hypertrophy. Mammary gland activity has also been described. An interesting type of behavior was reported by Boling, Blandau, Wilson, and Young (1939). Both sexes of newborn guinea pigs showed with great regularity a "heat response," when fingered, that was similar to that of an adult female in heat. The reaction could be elicited soon after birth and lasted for several hours, but it was strongest at the beginning of the period. This reaction occurred in newborn males and females whose mother had been ovariectomized during pregnancy (by the 50th or 55th day) and therefore was not dependent on hormones from the mother's ovaries.

The uterus of the fetal monkey is under prenatal estrogen influence, and the newborn monkey frequently bleeds for several days after birth. Uterine involution takes place within 1–2 months after birth (Engle, 1936). Other species furnish further examples of prenatal stimulation. The ovaries and testes of the fetal horse and donkey show unusual hypertrophy of interstitial cells (Cole *et al.*, 1933; Catchpole and Lyons, 1934). The fetal horse ovaries exhibit such remarkable growth that for a part of the fetal period they exceed the maternal ovaries in weight, but by birth they have regressed and may weigh only one-tenth of the maximal fetal weight. Both testes and ovaries in the fetal horse develop masses of interstitial cells, which appear at the 4–12-cm. stage. Fetal pig testes grow at a remarkably accelerated rate at a certain period and contain large amounts of interstitial tissue (Bascom and Osterud, 1927). These findings suggest that gonadotrophin has been acting on the fetal gonads, as it does on the gonads postnatally, and stimulating marked growth and interstitial cell hypertrophy. This interpretation is supported by the fact that great stimulation occurred in the ovaries of the pregnant mare at the same time that the fetal gonads showed their response. This indicated that there had been a high titer of gonadotrophin available to both mother and fetus; and the discovery of large amounts of gonadotrophin in the

maternal blood offered further evidence. Catchpole and Lyons (1934) concluded that there was a placental origin of this substance. The accelerated growth in pig testes (Bascom and Osterud, 1927) was found at a period just after detectable amounts of gonadotrophin had begun to appear in the fetal hypophysis (Smith and Dortzbach, 1929). There is no proof that this apparent correlation is actual, but it is an interesting observation that may have significance.

There is little doubt that prostatic hypertrophy in the human male fetus and newborn child is attributable to male hormone, and it is probable that this hormone comes from the fetal testes. Prostatic regression after birth indicates that the fetal testes are producing increased amounts of hormone in the late fetal period, presumably under the influence of gonadotrophic stimulation.

Finally, there is enlargement of the mammary glands in some newborn foals with the production of abundant secretion (Labat, cited by Courrier, 1945). This would involve the interaction of several hormones—estrogen, probably progestin, and mammotropin.

These various observations lead to the conclusion that fetal organs respond to prenatal hormone influence, as they do to postnatal stimulation. Some of the stimulation in the fetus occurs relatively early in pregnancy and some in later periods. The fundamental question then is whether the end-organs are responsive early enough in development so that if hormones are available they can play an active role in shaping the course of sex differentiation. Experimental work offers at least some of the answers to this question

C. EVIDENCE FOR REACTIVITY IN THE FETUS FROM EXPERIMENTAL STUDIES

Since Courrier (1924) reported the effects produced in the fetus by injection of folliculine into the pregnant guinea pig, many workers have studied the modifications of development that can be produced by this technique in rats, mice, cats, rabbits, guinea pigs, and hamsters. The most extensive research has been done on the rat by Greene, Burrill, and Ivy, on the mouse by Raynaud and by Turner, and on the guinea pig by Dantchakoff. Recently the hamster has been used by Bruner and Witschi (1946). In addition, the reactions of the reproductive tract of the pouch young of opossums, which are born in a very undeveloped and essentially "embryonic" state, have been studied by Moore and by Burns. Summaries and discussions of these studies for the rat, mouse, and opossum are given by Greene (1942), Raynaud (1942), Moore (1941*a*, 1944) and Burns (1942*a*, *b*, 1945).

The administration of androgens and estrogens to pregnant females and to pouch young opossums proved that end-organ reactivity was present in the fetal rat and mouse and the young opossum. The sex hormones had definite and striking effects, but they were not always the expected ones. For example, in rats, androgens stimulated the Wolffian ducts of females but did not inhibit the Müllerian ducts; estrogens stimulated the Müllerian ducts of males and inhibited the Wolffian ducts, but they also maintained the Wolffian ducts of females.

In opossums, androgen had only slight effects on male and female Wolffian ducts but stimulated the Müllerian ducts of males slightly and those of females to a marked degree. Estrogen stimulated not only the Müllerian ducts of males and females but also the Wolffian ducts, with those of the female more reactive than those of the male.

Some of these confusing results and the lack of sex-specific response were clarified by Burns (1942*a*, 1945), who showed that in opossums when the dos-

age of testosterone propionate was greatly reduced (5–8 γ daily) the Wolffian ducts of females were stimulated; and the development of a prostate gland was induced; but little or no Müllerian duct stimulation occurred. In male opossums this dose of androgen did not affect the Müllerian ducts.

It was a consistent finding in rats, mice, hamsters, and opossums that the administration of androgen stimulated the development of the male type of accessory organs, such as the prostate, in the female. Female prostate glands occur spontaneously in some rats and mice, but in the female opossum no anlage of the gland ever appears normally. These interesting results will be discussed below.

There was no effect of androgen or estrogen on the fetal gonads that could be interpreted as sex inversion in any species. There was, however, an important effect on the fetal ovary in that, with androgen treatment in the mouse and estrogen treatment in the rat, the ovaries of females which were treated prenatally and studied postnatally did not undergo normal ovulation and luteinization and the vaginal smears were abnormal (Raynaud, 1942; Turner, 1939; Greene *et al.*, 1940). This effect has also been produced by implantation of male hypophyses into newborn female rats (Pfeiffer, 1936) and by administration of androgenic and estrogenic hormones to very young female rats. Turner (1939, 1940) and Raynaud (1942) interpreted their results on the mouse as an effect upon the hypophysis, which is the explanation offered by Pfeiffer. This observation for the fetal ovary and hypophysis implies a fetal establishment of hypophysial sensitivity to sex hormone influence.

From their studies on rodents and opossums, the various workers formulated theories to account for normal sex differentiation or attempted to fit their results into the pattern of theories already suggested.

D. THEORIES OF SEX DIFFERENTIATION

Greene (1942) concluded that the dihormonic theory of sex differentiation (that is, stimulation of "homologous" and inhibition of "heterologous" structures by the respective sex hormones) is most logical but must be considered speculative. From studies on simultaneous administration of androgen and estrogen he demonstrated that if the hormones are combined the effects of estrogen can be nullified by adequate amounts of androgen, and vice versa.

Raynaud (1942, p. 424) interpreted the chain of events as "... gènes sexuels, substances inductrices primaires déterminant l'histogénèse des glandes génitales, substances sexuelles libérées par les gonades embryonnaires, et tenant sour leur dépendance la différenciation sexuelle du sinus uro-génital et des organes génitaux externes." He attributed the development of the Wolffian and Müllerian ducts to hormones from the appropriate gonads, and degeneration of the ducts to lack of appropriate stimulation.

Burns (1938) thought that the differentiation of the ducts was under direct control of the proper gonad component and that direct inhibition of the Müllerian duct by male hormone was also indicated. However, he concluded that the development of the external genitalia appeared to conform to a mon-hormonic type of control by male hormone.

Moore doubted the possibility that sex hormones could normally be acting as differentiating agents in either the male or the female, and he suggested that, in addition to the basic genetic sex-determining factors, ". . . . certain stimulating humoral substances issue from the entire somatic complex of the organism; and that these, rather than specific

secretions elaborated by the developing gonad, favor homologous duct development" (1941*a*, p. 41). He summarized his theory (1944, p. 140) thus:

> It is strongly suggested: that the control of sex differentiation, up to the stage of a well-established reproductive system, rests upon the genetic sex-determining factors present in the embryo which operate without the intervention of sex hormones; that, in fact, the differentiating gonads do not secrete hormones until a period considerably later than that during proper disposition of the two sets of sex ducts, in accordance with the genetic determination of sex; and that when sex hormone secretion begins, the reproductive system is thrown into unusual activities in growth and development characteristic of the puberal ages.

This implies that the genetic constitution, male or female, determines not only primary gonad differentiation but also the early development of Wolffian and Müllerian ducts and the presence and early development of the accessory reproductive glands proper to each sex. This is accomplished by the genetic constitution or by means of substances produced in every somatic cell and circulating in the blood stream of the respective sexes.

Wiesner (1935, 1936) propounded the mon-hormonic theory of embryonic differentiation from studies on the postnatal rat. He interpreted differentiation of the male on the basis of testis hormone from the fetal gonad but stated for the female (1936, p. 28):

> It may be concluded that the genetic constitution of the somatic cells of the zygotic female is sufficient to involve their female differentiation. The factors determining femininity become active directly in the somatic cells instead of expressing themselves via the gonad; they incite development of the Müllerian duct; they impress a female characterization upon the anlagen of the external genitalia; they may also be sufficient to effect the involution of the Wolffian system.

However, he qualified this somewhat (1936, p. 29):

> Male development appears to be the result of a reaction of a zygotic male with certain extracellular factors; female development appears to be the product of the reaction of zygotically female cells with intracellular factors or extracellular and extragonadic agents of unknown nature.

The theories of sex differentiation range from the di-hormonic theory, which depends upon the production of hormones in fetal gonads (with testis secretion produced earlier than ovarian hormone) to the mon-hormonic theory, in which differentiation in the male is stimulated by male hormone but female differentiation is dependent on the "genetic constitution," to a theory in which the genetic constitution determines the differentiation of both the male and female.

The experimental administration of hormones demonstrated that reactivity is present very early in development. The degree and kind of responsiveness depended upon the particular organ, the genetic constitution (♂ or ♀), the dose of the hormone, the simultaneous administration of both sex hormones, and the time in development when the hormones were introduced. The responses differed from species to species.

Some interesting and perhaps fundamental concepts have arisen from these studies. Critical periods (so called by Burns) existed in which some organs were very sensitive to inhibition; but if inhibition did not occur, the organ survived and could not be inhibited later. In the same way, specific responsiveness to stimulation was present for a limited period of time in some structures. For example, the urogenital sinus of the female opossum could be stimulated to form a prostate gland, which never occurs normally in the female opossum, if androgen treatment was given before the animal was 30 days old. After that age, androgen caused hyperplasia and meta-

plasia of the sinus epithelium (Moore, 1945).

The combined studies on hormone treatment of rodents and opossums have demonstrated a very complicated pattern of reactivity in fetal organs, at least as judged from the dosage levels of the hormones that were administered. As discussed above, some of the findings have supported the di-hormonic theory of sex differentiation and others have not. The results have been contradictory, and no clear-cut explanation of embryonic development on the basis of hormone effects has been possible from these experimental studies.

If sex differentiation is effected by the genetic constitution of the cell itself or by specific substances determined by the genetic constitution (produced in the somatic cells of males and females and released in the blood stream) it is pertinent to examine normal and experimental development to see whether there is evidence that would test this postulate.

E. APPLICATION OF SOME OF THE THEORIES TO DUCT DEVELOPMENT AND REGRESSION AND TO PROSTATE DEVELOPMENT

1. WOLFFIAN DUCT REGRESSION IN THE OPOSSUM

The opossum is born after a gestation period of only 13 days and is so undeveloped that the sex of the gonads cannot yet be determined. Moore (1941*a*) has described in detail the development of the reproductive tract in males and females. The Wolffian ducts present a very interesting regression, which does not seem to have been described for any other species. The mesonephros is a functional organ until about the 15th day of life, and the mesonephric, or Wolffian ducts, are well developed and serve as urinary ducts. However, by 15 days of age the Wolffian ducts of both sexes have begun to regress to a marked degree, as evidenced by a reduction in the size of the lumen. In the male the duct is greatly reduced in size at the posterior end, but it still joins the sinus. In the female the duct is interrupted and no longer joins the sinus. At 24 days of age the regression is still more evident in both sexes; in the male the connection with the sinus is broken, and in the female the duct is reduced to a solid cord for most of its length. In older male animals the Wolffian ducts enlarge, and the connection with the sinus is again established. The regression in the Wolffian ducts occurs at an age when Cowper's glands are well established and the prostate is developing.

Here we have the extraordinary situation of Wolffian duct degeneration in both sexes and a later building-up of the duct of the male. What can this mean in terms of the factors operating to cause this inhibition or lack of stimulation? If Wolffian duct degeneration occurs normally in females because of the genetic constitution (either expressed in the cell or by means of circulating substances), it is difficult to account for marked regression in a soma with male genetic constitution. If male differentiation rests upon male genetic constitution, there must be a delayed action in the cells or a threshold of reactivity in the Wolffian ducts that is above the level of whatever substance is circulating.

The results of injections of androgen into young opossums offer further evidence on this point (Moore, 1941*a*). With such treatment there was slight stimulation of the Wolffian ducts of males and females, but reduction in size of the duct was not prevented in the male, and the posterior connection of the duct with the sinus was in no case maintained even though androgen treatment was begun several days before regression would normally occur. This demonstrates a high

threshold to male hormone stimulation, as opposed to the thresholds for such structures as Cowper's gland and the prostate, which were stimulated by the treatment.

If the male genetic constitution controls development, it is apparent that different thresholds of reactivity, such as have been demonstrated by hormone treatment, must be operative.

2. MÜLLERIAN DUCT DEVELOPMENT IN THE RAT

The Müllerian ducts of male rat embryos begin to degenerate at about 17 days post coitum. When male Müllerian ducts were removed from embryos at 15 or 16 days post coitum, just before degeneration had begun, and were transplanted into rats that were 4–38 days of age, the Müllerian ducts did not degenerate, in many cases, but continued their development and differentiated into oviducts and uterus with glands (Moore and Price, 1942). The hosts were normal males or normal females, and in every case in which there was a well-developed male uterus there was also a testis in the graft. It was impossible to determine whether hormones from these sources had been stimulating the male Müllerian duct, but it was evident that there was no inhibiting influence. How soon hormone influence might have reached the implant is a question. The important point here is that the male genetic constitution cannot be responsible for causing degeneration directly, because the ducts developed when they were removed from their embryonic environment. Their normal degeneration in the embryo must depend upon the presence of an inhibiting influence or the lack of a stimulating one; but the tendency to degenerate cannot be considered intrinsic in the cells because of their male genetic constitution.

3. PROSTATE GLAND DEVELOPMENT IN FEMALE OPOSSUMS AND RATS

The female rat sometimes develops a prostate gland, which resembles histologically the ventral lobe of the male prostate, and the character can be established in a strain by inbreeding (Witschi *et al.*, 1938, 1939). This character cannot be considered a specifically male character in the rat, but the female opossum never develops an anlage of the gland at any stage in embryology, and the induction of such a gland could therefore be considered "masculinization" of the female.

Treatment of the pouch young female opossum with androgen causes the development of a prostate gland, which resembles that of the male in every way except in size (Burns, 1939; Moore, 1941*a*). When the gland is once induced to develop from the sinus, it continues to grow and differentiate after androgen treatment is discontinued and reaches a stage that is equivalent to that of a 100-day-old male (Moore, 1945).

The prostate of the opossum can be considered a specifically male character which should develop because of male genetic constitution. Male hormone exactly simulates in every respect the postulated action of the genetic male organism and induces a prostate in the female. What factor causes continued development of the female prostate after androgen treatment ceases? If the female organism produces substances that are responsible for developing the female prostate (Moore, 1945), then the differentiation of a typical male character depends upon the female genetic constitution.

In strains of rats which have a very low percentage of spontaneously occurring female prostates, such glands can be induced in the female fetus by injection of androgens into the pregnant female (Greene, 1942; Mahoney, 1942). The induced female prostate continues develop-

ment after birth without further treatment and assumes a functional-appearing histological condition resembling that of the male, for a limited period of time. This is similar to the development attained by normally occurring female glands (Price, 1939; Mahoney, 1940) and has been shown to be dependent upon the presence of ovarian and/or adrenal androgens (see Price, 1941, 1942, 1944*a*, for discussion). Here hormones from two sources in the female have been effective in stimulating female prostate differentiation; but how early these hormones can act on development has not been demonstrated.

4. MALE PROSTATE TRANSPLANTS (RAT)

Male rat prostate tissue, taken from very young postnatal males and transplanted into adult females (virgin, pregnant, or lactating) or males, grew and differentiated and was maintained in a well-developed histological condition similar to that of the gland in a normal adult male (Price, 1941, 1942). Hormone from the ovary (designated as "ovarian androgen") stimulated the grafts in female hosts; and it was shown that male prostate tissue had a lower threshold to ovarian androgen than did the female prostate gland in the hosts. In addition, there was a great increase in ovarian androgen in pregnant and lactating females. In adult male hosts, testis-secreted male hormone provided the stimulation. However, if such tissue was put into adult spayed females or castrated males, no further growth or differentiation occurred; and fifty such grafts recovered from gonadectomized hosts contained only small, round prostate acini at about the stage of development that they had reached at the time of transplantation. Male prostate tissue was incapable of self-differentiation, that is, it was not carried on in development by virtue of its male genetic constitution. It was, however, highly responsive to androgens from the testis and the ovary.

These examples show that is is difficult to interpret sex differentiation, i.e., duct development and regression and accessory gland stimulation, on the basis of a postulate which calls for a fixed action of the genetic constitution in each cell concerned with the differentiation. If, on the other hand, sex differentiation is effected by substances produced in the somatic cells of males and females, orderly sex differentiation must depend upon a series of differences in threshold response of the end-organs, and these differences must be of the type that has been demonstrated by hormone treatments.

F. THE RELATION OF HORMONES TO THE DEVELOPMENT OF PRIMORDIA AND TO EARLY DIFFERENTIATION

It is recognized that hormones cannot affect an organ or structure unless the ability to react is already present and that hormones could never, for example, stimulate the development of the outgrowths of the prostatic anlage from the urogenital sinus unless specific organization and sensitivity existed. Weiss (1939, p. 442) has stated the case very clearly:

> Applied to the organism, this means that hormones being diffusing agents could never produce manifest differences among parts unless the parts were already latently distinguished from one another, at least so far as specific and selective sensitivity to the hormone is concerned. Hormones expose and modify existing differences but they cannot create new ones. Thus they cannot be primary factors of organization. But, once the fundamental organization has been laid down and parts have assumed distinctive properties including hormone sensitivity, then, indeed, hormones rise to great prominence as instruments of secondary reintegration in that they bring the individual parts back under a common control.

The establishment of such organization and sensitivity is a function of gene action, and the introduction of hormones experimentally has demonstrated that the ability to respond in specific ways is present very early in development. In the sinus, for example, sensitivity is apparent just before or at the time that the primordia of the prostate develop normally in the male. The fact that male hormone can induce all the lobes of the prostate in the female rat so that the female urogenital sinus resembles exactly that of the male seems to suggest that the primordia in the male may be induced by male hormone action.

Mahoney (1942) concluded that the rat prostate had an early period of self-differentiation, but Bruner and Witschi (1946), as a result of their studies on induction of prostate and bulbo-urethral glands by androgen treatment in female hamsters, stated:

> The fact that intensification to and above the threshold level is brought about by androgens suggests a possible corresponding role of hormones in the normal course of development of these glands in the male. It seems conceivable, though actual proof is still lacking, that during the earlier steps of histological differentiation minute amounts of androgens are liberated at specific foci.

On the other hand, if the "genetic constitution" is thought of as determining differentiation, it must be recognized that the genes themselves do not determine directly the presence or absence of a character. Wright (1945, p. 291) has emphasized the point in this way:

> Even physiologists sometimes attribute a character partly to physiological factors, partly to heredity, as if heredity could operate by some sort of sympathetic magic, independently of physiological channels. The attitude of physiological genetics is that characters are determined 100 per cent by physiological processes, but that genes are the ultimate internal physiological agents.

> Genes can be thought to act directly only on the metabolic processes in the protoplasm immediately surrounding their positions in the cells which contain them. They can affect morphological characters only by determining one or another reaction of cells to local conditions.

It is quite possible that genes might produce the early differentiation of the parts of the reproductive tract in the same way that differentiation of other organs of the body is effected (see Wright, 1945). Specifically, the development of prostatic primordia from the male sinus (after organization was established) might be controlled entirely by the genes through local increase in mitosis, etc.; but, since an exact replica of early male development can be induced in the female by male hormone, it is possible that male hormones may be the factor in bringing out male development also, especially since fetal testes have been shown to be capable of secreting male hormone. It may also be possible that, once the sinus is organized and sensitivity is established, nonspecific agents of various kinds might be able to stimulate prostate development. Such a finding would, however, not answer the question of whether normal development is controlled by male hormone and would not exclude that possibility.

If male hormone controls the development of the male prostate gland, what can be said of the prostate which occurs spontaneously in some females? If hormones control the development of the female prostate, it necessitates the assumption that ovarian or adrenal androgens are available and that the ventral portion of the sinus in the females that develop a prostate (only the ventral lobe occurs spontaneously) has a lower threshold to stimulation than in the females that have only small abortive primordia of the gland. In this regard,

Mahoney (1942) showed that the ventral portion of the sinus in female rats of the prostate strain is more sensitive to androgen than that part of the sinus in females of the nonprostate strain. The genetic difference in the two strains was expressed in specific differences in sensitivity in the parts of the sinus. There is evidence that androgens are increased in pregnant female rats (Burrill, 1942; Greene, 1942), and the urogenital sinus of the fetus may even be stimulated by maternal hormones.

Regardless of whether gene action or sex hormone action produces the prostatic anlage and maintains the Wolffian and Müllerian ducts in their respective sexes, it need not be assumed that all further growth is hormone-conditioned any more than that all growth in other organs of the body is so governed. Once the presence of a structure is assured and the critical period passed (and experimental evidence indicates that small amounts of hormone are effective in early critical periods of development), at least part of the subsequent growth and development must be independent of hormone action, even though hormones may be present and producing stimulation.

The role of sex hormones in early differentiation needs to be clarified by procedures in which the sources of sex hormones are removed. Embryonic castration would dispense with the source of testis hormone in the male. Embryonic spaying cannot assure the absence of female hormone in the female fetus of placental mammals because of the distribution of estrogen (from the placenta or the maternal circulation) in fetal organs. The male fetus would have these same sources of female hormone. Removal of fetal gonads presents great technical difficulties, but some successful experiments have been reported.

G. RESULTS OF "EMBRYONIC CASTRATION" IN THE OPOSSUM, RAT, AND RABBIT

Moore (1941*b*, 1943) removed the gonads from male and female pouch young opossums 20 days of age and a little older and studied the development of the prostate gland of males and the uterus of females. The opossum of this age can still be considered "embryonic." From histological studies, Moore concluded that gonadectomy did not prevent the further development and differentiation of the prostate and the uterus and that no effect of gonad removal was apparent. His operations, however, were performed several days after the beginning of prostate formation and at an age when Müllerian ducts in males and Wolffian ducts in males and females had undergone considerable regression.

Wells (1946) castrated fetal male rats at about 19 days and 20 hours post coitum and killed them 2 days later. He reported a reduction in the volume of the seminal vesicles and bulbo-urethral glands. As in the opossum, the testes were removed after the anlagen of the accessory reproductive organs were formed and regression of the Wolffian and Müllerian ducts was well advanced. No conclusions can be drawn as to whether the testis-secreted hormone was necessary for the formation of the primordia of the accessory glands.

Jost (1947*a*, *b*) succeeded in castrating and spaying fetal rabbits by 21 or 22 days post coitum, before the degeneration of Wolffian ducts in females and Müllerian ducts in males. The animals were autopsied about 27 or 28 days post coitum (gestation is 32 days). Castration of males resulted in degeneration of the Wolffian ducts, lack of seminal vesicle formation, and reduction in the number of prostatic outgrowths and the size of the gland; but the Müllerian ducts were

maintained. Unilateral castration did not produce any effects upon the fetus. Spaying of females did not cause any change in sexual differentiation; but, when gonadectomy was performed at the youngest age, the Müllerian ducts of the spayed females were smaller than those of controls. Castrated males resembled spayed females except that the genital cord was shorter and prostate anlagen were found only in males.

Jost interpreted his results to mean that the Müllerian ducts evolve and differentiate, whatever the sex of the host, unless they are inhibited by male hormone; that the Wolffian ducts atrophy if they are not maintained by male hormone; and that the prostate and external genitalia are also under the control of testis hormone.

These experiments are preliminary ones, but if they are substantiated they offer a most interesting and fundamental contribution in support of a mon-hormonic type of sex differentiation.

H. CONCLUSIONS

1. Estrogen, progestin, gonadotrophin, mammotropin, and androgen have been found in the blood and organs of the fetus or in the urine and blood of newborn animals of several species. Some of these hormones are undoubtedly of placental or maternal origin, but there is evidence that fetal gonads secrete sex hormones that circulate in the blood of the fetus.

2. Studies of the gonads, uterus, vagina, mammary glands, and prostates of fetal and newborn animals have shown that these organs have been responding to prenatal hormone stimulation under normal conditions.

3. Experiments on fetal rodents and on pouch young of the opossum have demonstrated that the Wolffian and Müllerian ducts and urogenital sinus are reactive to hormone treatment very early in development. However, the expected results on the basis of sex hormone stimulation of "homologous" characters and inhibition of "heterologous" characters have been obtained in only some cases, and the results are contradictory. There is evidence that some of the lack of sex-specific response in the opossum is due to the level of dosage that was administered.

4. Several theories of sex differentiation have been proposed: (1) the di-hormonic, (2) the mon-hormonic, and (3) a theory postulating that sex differentiation is attributable to the specific genetic constitution (male or female) without the intervention of gonad-secreted hormones.

5. Crucial experiments in embryonic castration in the rabbit have been performed by Jost (1947*a*, *b*). His results are explainable on the basis of mon-hormonic development. Although the mon-hormonic theory explains his experimental findings, it is not supported by all the results obtained with hormone treatments. It would be expected that embryonic castration would provide a sounder basis for the interpretation of sex differentiation than the administration of hormones.

6. No theory yet suggested is applicable to all the results obtained. Species differences in response may indicate that there are also species differences in the means by which sex differentiation is effected.

III. Postnatal Development to the Completion of Puberty with Special Reference to the Rat

The role of hormones in determining the prenatal development of the reproductive tract is still a question, but there is no doubt that hormones control its postnatal growth and differentiation.

The chain of events in relation to the development of the reproductive tract may be visualized as follows: pituitary gonadotrophic hormone, with the follicle-stimulating and the luteinizing factors, stimulates the gonads to develop and produce mature sperm and ova and to secrete their sex hormones, which control the growth and differentiation of the accessory organs of reproduction, such as the prostate and seminal vesicles of males and the uterus and vagina of females.

The pituitary, which controls the other endocrine glands through its production of gonadotrophic, thyrotrophic, and adrenocorticotrophic hormones (among others) is, in turn, influenced by the hormones secreted by the gonads, the thyroid, and the adrenal and by such environmental factors as diet and light.

Although the development of the gonads depends upon the presence of gonadotrophic hormone and that of the accessory reproductive organs upon sex hormones, the rate at which development proceeds and the age at which functional differentiation is reached are determined also by the reactivity of the end-organs themselves. This reactivity is not a static property of the cells and organs but is undoubtedly influenced by many factors.

To understand the interrelationships of the factors which operate to bring the reproductive tract to a functional state at puberty, it is necessary to determine the level of hormone production as age advances and the changes in reactivity of the end-organs. Several such studies have been made on the rat, which will serve as an example to illustrate development. However, it must be recognized that there are species differences and that a factor which is of great importance in one species may have less significance in another.

The growth of the reproductive tract is limited, in the first place, by the kinds and amounts of hormone present.

A. AVAILABILITY OF HORMONES TO THE DEVELOPING ORGANISM

1. MATERNAL HORMONES THAT REACH THE SUCKLING RAT IN MILK

At birth the newborn animal is freed from any hormone influence that may have been coming from the placenta but is dependent upon milk, and milk has been shown to contain estrogenic and gonadotrophic hormones. The findings in relation to hormones in milk have been discussed recently (Price, 1947). To summarize, estrogen has been found in the milk of oestrous goats and pregnant cows and in human colostrum and milk for 9 days after parturition. Gonadotrophin has also been reported in human colostrum and milk, and it, too, disappears from the milk by about 6 days postpartum. It would not be expected that these hormones would have any important effects on development in the newborn child. However, some of the estrogen and gonadotrophin found in the urine of newborn children for a few days after birth may come from the mother's milk and may have been producing slight effects.

In the rat there is evidence that maternal hormones reach the suckling young in effective amounts. Removal of the ovaries of lactating females reduced the weights of the prostate and seminal vesicles of the suckling males in their litters and proved that maternal ovarian androgens were exerting an influence on growth (Price, 1947). No effect was observed in suckling males after 14 days of age. Marx (1945) induced lactation in young suckling female rats by administration of testosterone propionate. When the same treatment was given to rats after weaning, growth of the mam-

mary glands resulted, but no lactation occurred. Marx postulated that estrogen and/or pituitary lactogenic hormone were supplied to the suckling young through the mother's milk.

These studies suggest that maternal ovarian androgen, estrogen, and hormone from the anterior pituitary reach the suckling rat. Other hormones from the pituitary, thyroid, and adrenal may also be carried in the milk. It is not known whether such maternal hormones play a significant role in early postnatal development, but the possibility of their presence must be recognized in any interpretation of experimental results in suckling animals. It is conceivable that in an animal such as the opossum, which is born in an undeveloped state, maternal hormones in the milk might be of importance in early differentiation.

2. HORMONE PRODUCTION IN RELATION TO AGE IN THE ENDOCRINE GLANDS OF YOUNG RATS

a) HYPOPHYSIAL HORMONES

1) *Gonadotrophic hormone.*—Clark (1935*a*) studied the gonadotrophic potency of the hypophyses of male and female rats from birth to 7 months of age by implanting the glands in immature mice. Gonadotrophic hormone was found in the hypophyses of newborn rats, which showed that the fetal rat hypophysis is already functioning. She determined that there were important age and sex differences in potency. In the male the potency of the gland increased gradually to a peak at puberty (30–60 days), which was maintained in older animals. In the female between 13 and 20 days of age there was a sharp rise in the gonadotrophic content of the pituitary to a level that was not surpassed at maturity.

A comparison of the sexes showed that from birth to 20 days of age the gonadotrophic content of the female hypophysis was markedly greater than that of the male, but, by 60 days, the male usually exceeded the female. In older rats the male hypophysis was definitely superior in gonadotrophic potency. Clark concluded that the marked differences in potency of the hypophyses of very young males and females might depend upon a precocious secretion of male hormone, which inhibited the male hypophysis. In further studies (Clark, 1935*b*), male and female rats were gonadectomized at birth, and their hypophyses were tested at 16–18 days of age. A negligible sex difference was found, indicating that the assumption was correct.

The results of McQueen-Williams (1935) corroborated the findings of Clark and added new data. In 1–23-day-old rats the gonadotrophic content of the hypophysis of the male was much below that of the female; in 27–30-day-old rats a great prepuberal increase in gonadotrophic potency had occurred in the male hypophysis, which then approximately equalled the female; in 35–38-day-old animals a sudden prepuberal drop was apparent in the potency of both male and female hypophyses (the pituitaries were heavier than at the previous age, but they had lost two-thirds of their potency in 1 week); in 42–44-day-old rats the male remained the same, but gonadotrophin was lower in the female hypophysis. In older animals the male hypophyses were superior in gonadotrophic activity.

The finding of a high titer of gonadotrophic hormone in the hypophyses of very young animals does not necessarily indicate that the hormone is being released in the same quantities to the organism, since production and release are not necessarily correlated. However, if production of hormone in the gland does correspond to release, there is a marked increase in the amount of gonadotrophic

hormone available to male and female rats at specific periods in development.

Research on other species has demonstrated that there are age and sex differences in the gonadotrophic potency of the pituitary, but it is a general finding that the pituitaries of young animals are high in potency (see Smith 1939, p. 954; Reece and Turner, 1937, p. 29).

2) *Thyrotrophic hormone.*—Turner and Cupps (1939) studied the thyrotrophin content of the rat anterior pituitary during growth and found that in both sexes there was a rise in potency during the period of rapid growth. The rats were grouped according to weights, not ages; and a marked increase in thyrotrophin concentration occurred between weights of 75 (the youngest rats that were examined) and about 150 gm. As judged by the weights and ages given by Kibler and Brody (1942) in their study of metabolic rate, the increase in thyrotrophin occurred between 34 and 50 days of age. The level of thyrotrophic potency fell later, at the time when body growth slowed. The male hypophysis contained a significantly higher concentration of thyrotrophic hormone than the female in every weight group.

In the mouse, Adams and Mothes (1945) showed that the thyrotrophin content of the pituitary of both sexes increased from prepuberty at 26–27 days to puberty at 40–41 days and decreased when the mice were fully mature at 82–83 days. The thyrotrophic potency of the female pituitary was greater than that of the male at 26–27 days and 40–41 days, but it was less at 82–83 days.

Adams (1946) reviewed the literature on the potency of thyrotrophic hormone of the pituitary. The relation of age to thyrotrophic potency has been studied in rats, rabbits, guinea pigs, cattle, swine, and humans. Adams states: "With respect to age, the assays have indicated an increase in potency from prepuberty to puberty, a period of rapid growth, followed by a decrease after sexual maturity has been established."

b) GONAD HORMONES

1) *Androgenic hormone.*—The testes of the male rat secrete hormone before birth (Wells, 1946). Many studies on castration of very young postnatal animals have demonstrated that the growth and development of the accessory reproductive organs are inhibited in the absence of the testes. If gonadectomy is performed on 2-day-old rats, the weights of the prostate and seminal vesicles are significantly reduced as compared with the normal by 8 days of age (Price, 1947).

The rate of secretion of testis hormone has been estimated by castrating rats at different ages and determining the dose of androgens that would maintain the normal growth of the accessory reproductive organs for 20-day periods. Moore and Price (1938) found that a larger daily dose of testosterone propionate was necessary to keep the seminal vesicles growing at the normal rate between 40 and 60 days of age than that required between 30 and 50 days of age and that the maintenance dose was still higher between 50 and 70 days.

This study has been extended to younger rats, using a shorter period of maintenance of gland growth, so that the secretion of testis hormone could be estimated at closely graded ages (Price, unpublished). Results on over 100 rats showed that normal prostate and seminal vesicle growth could be maintained from 8 to 14 days by a little more than 1 γ of testosterone propionate daily; from 20 to 26 days by 5 γ; from 30 to 36 days by about 10 γ; from 50 to 56 days by 25 γ; and from 70 to 76 days by 50 γ. This is only an approximate estimate; but, if these values represent the rate of normal

hormone secretion, there is a gradual rise in hormone production.

A gradual and uniform increase in testis hormone production in relation to age in the young bull was reported by Hooker (1937). From his assays of testis extracts he concluded that there was no great change in hormone production associated with puberty and the attainment of sexual maturity. Studies on the urinary excretion of androgen in the human male have shown that there is a gradual increase in excretion of the hormone with age in the child, and a marked rise, according to some workers, at puberty (see Burrows, 1945, p. 169).

2) *Estrogenic hormone.*—There is no experimental evidence that the rat ovary is secreting detectable amounts of estrogen in the first week of life. Wiesner (1935) reported that there was no change in uterine size or in prepuberal development when the ovaries of the rat were removed soon after birth. Other work has shown that there was no significant difference in uterine weights between spayed females and normal littermates at 8 days of age (gonadectomy at 2 days), but by 14 days of age there was a highly significant difference (Price, 1947). This indicates that the ovary secretes little or no hormone in the first week of life but produces large enough amounts in the second week to cause a material increase in the weight of the uterus. No research estimating the amount of female hormone that is produced by the ovary at different ages is available, but from Figure 1 it can be seen that uterine growth is slow until about 36 days of age. The rat uterus is responsive to estrogen administration in the first week of life (Price and Ortiz, 1944), so that its slow normal growth seems to reflect a low level of estrogen production by the ovary. Between 36 and 56 days of age, however, rapid growth occurs, which may be partly attributable to increased production of female hormone coincident with the beginning of ovulation and establishment of oestrous cycles. Studies on the human female indicate that urinary excretion of estrogens increases at puberty (see Burrows, 1945, p. 170).

c) ADRENAL HORMONES

1) *Androgenic hormone.*—There is evidence that the adrenal gland of rats is capable of producing androgenic hormone which influences the prostate gland of males and females. Howard (1938) first suggested that androgen from the adrenal cortex might be responsible for the marked development of the prostate gland that was found in young castrated male rats (Price, 1936). Since that time a series of reports by Howard and Burrill and Greene have dealt with adrenal androgen production in the rat and mouse (see Parkes, 1945, p. 227). In the young rat it has been established that the adrenal of gonadectomized males and females is capable of producing detectable amounts of androgen until about 35 days of age. The level of production in the normal animal and the possible significance of this androgen in development are not known.

Experiments on early gonadectomy in inbred strains of mice have shown that nodular hyperplasia of the adrenal cortex occurred in association with the production of androgen, which affected the accessory reproductive organs of males and females (Woolley, Fekete, and Little, 1941; Woolley and Little, 1945*a*, *b*). These effects were observable after several months, and therefore they provide no suggestion as to whether the adrenals were secreting androgen in the very young animal or whether the normal mouse produces effective amounts.

2) *Estrogenic hormone.*—The rat adrenal also secretes estrogenic hormone

which apparently stimulates vaginal opening in the absence of the ovaries (Wade and Haselwood, 1941). A number of workers have observed that in ovariectomized rats (and mice) that are injected with threshold doses of estrogens there is cyclic cornification in the vagina. Zuckerman and his collaborators have thoroughly investigated this problem and have suggested that the adrenal is responsible for the fluctuation in vaginal sensitivity to estrogenic stimulation. These experiments and others dealing with the injection of corticotrophic extracts in ovariectomized rats and the resulting effects on the uterus and vagina are reviewed by Parkes (1945, pp. 229–31).

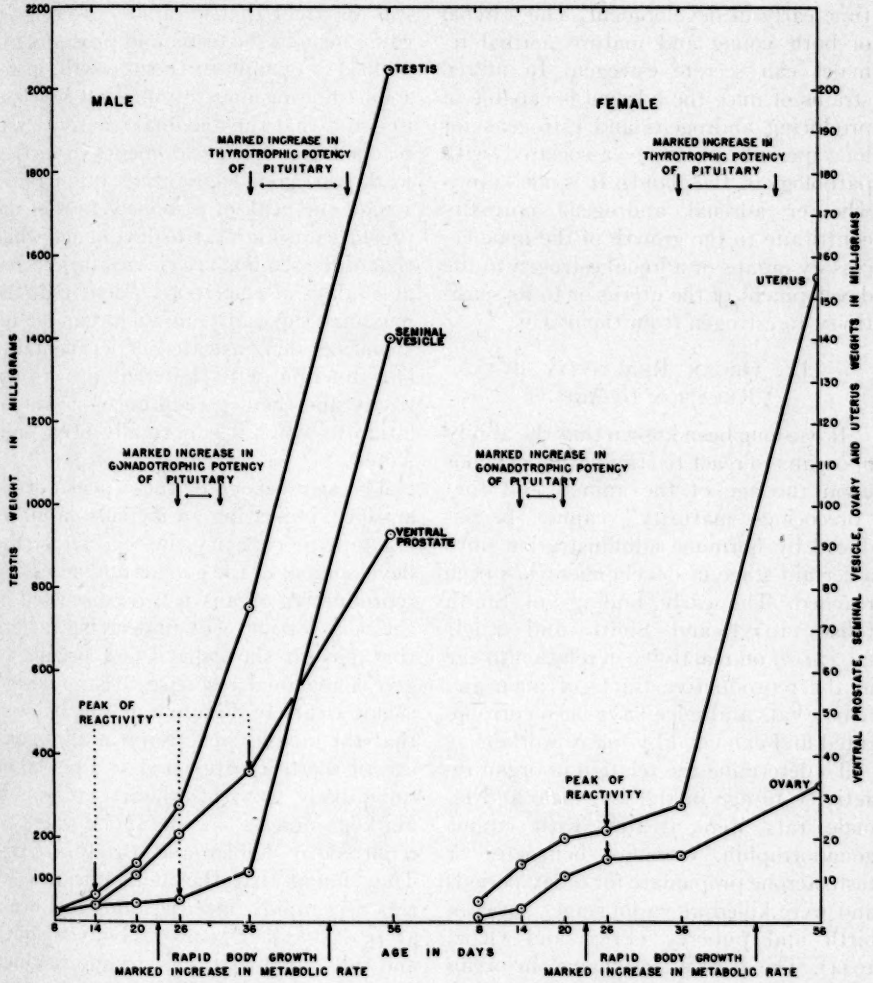

FIG. 1.—Growth of the gonads and accessory reproductive organs of male and female rats in relation o various factors. Weights based upon data for 200 male and 200 female rats.

Woolley and his co-workers have demonstrated that gonadectomy in certain inbred strains of mice results not only in androgen production by the adrenal but also in estrogen production in both males and females.

From these studies it is apparent that the rat adrenal can produce effective amounts of androgen in gonadectomized males and females for a limited period of time early in development. The adrenal of both young and mature normal females can secrete estrogen. In inbred strains of mice the adrenal is capable of producing androgens and estrogens for long periods of time (associated with pathology of the gland). It is not known whether adrenal androgens normally contribute to the growth of the male accessory organs or adrenal estrogen to the development of the uterus or to its sensitivity to estrogen from the ovary.

IV. Organ Reactivity in Relation to Age

It has long been known that the ability of organs to react to stimulation depends upon the age of the animal and that "precocious maturity" cannot be induced by hormone administration until a certain stage of development has been reached. The early findings of Smith (1926, 1927) and Smith and Engle (1927*a*, *b*) on reactivity in relation to age in the reproductive tracts of male and female rats and mice have been corroborated and extended by many workers.

To determine the relation of organ reactivity to age in the rat, male and female rats were treated with equine gonadotrophin, estradiol benzoate; or testosterone propionate for 6-day periods and were killed at various ages between birth and puberty (Price and Ortiz, 1944). The percentage change in organ weight as compared with the normal controls was determined, and the peak of responsiveness was established for the gonads and accessory reproductive organs. It was found that reactivity increased with age in all the organs and that the greatest ability to respond (as judged by percentage increase in weight above the normal) was reached in the testis by 14 days of age, in the prostate by 18 days, and in the seminal vesicle, ovary, and uterus by 26 days of age. It was apparent that in rapidly growing organs, such as the testis and prostate, the ability to exhibit further growth, in response to exogenous stimulation, was limited and that the maximal reactivity was reached early in development. In further work on gonadectomized rats (Price, 1944*b*) the peak of responsiveness of the prostate was found at 36 days of age, while that of the seminal vesicles again occurred at 26 days of age. It is evident that the maximal capability of an organ to respond, as demonstrated experimentally, is a function of its inherent growth capacity and the degree of hormone stimulation to which it is normally being subjected.

The significance of these peaks of reactivity, indicating an increase in inherent growth capacity, in relation to the development of the gonads and accessory reproductive organs will be discussed in the next section. The underlying factors that govern the capacity of organs to give a maximal response to stimulation is not clear. In Figure 1 it can be seen that the greatest reactivity in all organs except the testis appeared at a period of rapid body growth, when there was a marked increase in metabolic rate, as reported by Kibler and Brody (1942). They found that the metabolic rate of rats rises rapidly after weaning to a peak at 45 days of age (100 gm. body weight) and falls after that, the decline tending to parallel the decline in percentage growth rate.

It is possible that in a period of normally high metabolism the organs may utilize hormone to better advantage and thus increase in responsiveness. The rapid increase in metabolic rate may be correlated with thyroid activity due to the increasing thyrotrophic potency of the pituitary (Turner and Cupps, 1939). There is considerable evidence that hyperthyroid and hypothyroid states influence the ability of organs to respond to hormones (Fluhmann, 1934; Smelser, 1939; and others). As discussed above, Zuckerman has postulated a sensitizing effect of adrenal hormone on the uterus. Whatever the mechanism, increase in responsiveness in some organs seems to bear a definite relation to the growth curve of the organ.

The significance of the marked increase in reactivity with the approach of puberty was first stressed by Hooker (1942), who found a great increase in sensitivity of the seminal vesicles of gonadectomized rats to testosterone administration at 40–60 days of age.

Ortiz (1947) found that the reproductive organs of the hamster also show increasing reactivity with age. The greatest responses, as judged by percentage increases in weight over the normal, were observed at 16 days in the testis and male accessory organs, 36 days in the ovary, and 6 and 10 days in the female accessory organs. Oestrous cycles begin at about 30 days of age in the hamster, so that the greatest reactivity in the ovary comes after puberty has been reached.

Wilson and Young (1941) have studied sensitivity to estrogen in female rats and guinea pigs by means of experimentally induced mating responses. Sensitivity increased with age, and by 30 days both the rat and the guinea pig had attained a high sensitivity, with the estrogen threshold for mating response comparable to that of adults.

V. Organ Growth and Differentiation in Relation to Age, Hormone Stimulation, and Organ Reactivity

In the preceding sections it has been shown that the gonadotrophic and thyrotrophic potencies of the pituitary and the metabolic rate of the growing animal increase at certain periods in development and that the gonads and accessory reproductive organs have an increasing ability to respond to stimulation as age advances. In Figure 1 are plotted the growth curves of the gonads, prostates, seminal vesicles, ovaries, and uteri of groups of albino rats (from the colony in this laboratory) from 8 to 56 days of age, in relation to all these factors.

The normal growth of the testes is rapid, since these organs double or more than double their weight at each 6-day period up to 36 days, when their growth is a little slower. The increased gonadotrophic potency at 27–30 days is not correlated with a period of more rapid testis growth than has been evident at the younger ages. The peak of responsiveness at 14 days of age does not seem to bear a significant relation to growth, since the weight increase in the 8–14-day period is as great as at any subsequent period.

If the experimental estimate (given above) of the rate of testis hormone production at the various ages corresponds to the actual rate of secretion, there should be a gradual increase in the amount of male hormone available to the organism, without any marked rise that would indicate intense gonadotrophic stimulation of hormone production by the testes. The findings on the prostate reported below substantiate this postulate.

The histological differentiation of the testis is a gradual process culminating in the appearance of sperm heads in the seminiferous tubules at about 36 days of

age and the presence of mature sperm by 56 days. There is no profound change in histology that would indicate increased gonadotrophic stimulation at the 27–30-day period.

The prostate gland also grows rapidly and, as with the testis, doubles or more than doubles its weight at each 6-day period to 36 days of age. Further increase in weight to 56 days is not quite so rapid. The curve of increase in prostate weight suggests rapid growth in response to adequate stimulation from male hormone but supports the idea that there is no sudden increase in male hormone production. The peak of prostate reactivity at 36 days of age does not seem significant in regard to normal weight increase, since the subsequent growth rate is unaffected.

Histological differentiation of the prostate gland is reached very early in development. The cell light areas, which are indicative of a normal "functional" gland, typical of the adult condition, are found by 12–14 days of age. Further development is rapid, and by 26 days of age the prostate resembles that of the adult except for the size of the acini. The growth and differentiation of the prostate indicate that it has a very low threshold to male hormone (which has also been shown experimentally, see Price, 1944*b*) and grows under the influence of a gradually increasing titer of male hormone. When the level of hormone reaches the threshold for differentiation, light areas appear in the cells.

The prostate growth appears to correlate closely with testis function, but it must be admitted that the adrenal is a possible source of supplementary stimulation for the prostate in the early period of development. A part of the rapid prostate growth from 8 to 14 days of age is definitely attributable to stimulation from ovarian androgen in the mother's milk.

The seminal vesicles present a different type of growth from that of the prostate. They double their weight in the period from 8 to 14 days of age (maternal ovarian androgen supplies part of the stimulus for this growth), but their subsequent weight increase is very small until 26 days of age, when more rapid growth begins, and the increase in weight between 36 and 56 days of age is striking. Since the studies on the testis and the growth of the prostate have not indicated that there is a marked increase in testis hormone production at 20 days, the speed-up in growth of the seminal vesicle seems to be correlated with its increased ability to give a maximal growth response to stimulation at that age.

The seminal vesicle differentiation proceeds slowly, and the secretion granules, which are used as a criterion of the adult type of cell differentiation, do not begin to appear until 36 days of age. The seminal vesicles require a much higher level of androgen to produce differentiation of the epithelium than does the prostate. It appears that histological differentiation of the seminal vesicles occurs when the male hormone level in the developing animal reaches the seminal vesicle threshold for differentiation. This is the probable explanation, since experiments at various ages on the reactivity of the seminal vesicle epithelium to minimal effective doses of testosterone propionate have not demonstrated any increase in sensitivity at this or at any age (Price, 1944*b*).

The curve of ovarian growth is a broken one. From 8 to 14 days of age, the weight more than doubles, and between 14 and 20 days the ovary triples its weight. At that age, growth becomes slower, up to 26 days; a plateau is reached between 26 and 36 days of age; and weight again increases between 36 and 56 days. This same type of curve for ovarian weights of the rat is given by

Donaldson (1924, p. 221), and the plateau appears at the same age-period (judged by the body weights in his graph) as in the rats in our colony. The rapid growth between 14 and 20 days of age appears to correlate with the rapid increase in gonadotrophic potency of the pituitary in that period, but growth of the ovary does not continue at a rapid rate to 26 days in spite of the continued maintenance of high pituitary potency. The sudden prepuberal reduction in gonadotrophic potency of the pituitary that is found in 35–38-day-old rats suggests that the plateau in ovarian growth may actually represent a reduction in the amount of available gonadotrophin. The peak of ovarian responsiveness is reached at 26 days of age (between 20 and 26 days the ovary is capable of great response to exogenous gonadotrophic stimulation), but this peak does not seem to bear a direct and significant relationship to normal ovarian growth.

Ovarian differentiation proceeds slowly at first, but after antra develop in the follicles there is rapid increase in follicular growth. The period from 20 to 26 days is one of marked increase in follicular diameters and in the percentage of follicles with antra (Lane, 1935*a*). It is beyond the scope of this study to attempt to analyze ovarian differentiation in terms of secretion and release of the follicle-stimulating and luteinizing factors of the pituitary. Lane (1935*b*) and Lane and Greep (1935) have made such an analysis and have advanced an interesting theory.

By 56 days of age the ovary is mature in many females, ovulation has taken place, the vagina is open, and oestrus cycles have become established.

It is difficult to estimate the production of female hormone by the ovary except in relation to uterine growth. The uterus grows rapidly between 8 and 14 days of age (it almost triples in weight), more slowly between 14 and 36 days, and very rapidly between 36 and 56 days of age. The peak of reactivity of the organ appears at 26 days and may bear a significant relationship to the abrupt rise in weight between 36 and 56 days of age, although much of this growth is probably due to increased amounts of female hormone. No marked differentiation of the uterine mucosa occurs until the time when oestrous cycles begin.

VI. Summary

A study of the factors which influence the postnatal growth and differentiation of the gonads and accessory reproductive organs may be summarized as follows:

1. Testis growth is rapid in the prepuberal period and gives no evidence of a sudden marked increase in gonadotrophic stimulation at any time. Testis hormone production increases gradually and fairly rapidly with age. From the studies of Clark (1935*a*, *b*) it is apparent that the reciprocal relationship between the testis and the pituitary is established very early.

2. Prostate growth is rapid and indicates that the gland is responding to a gradually increasing titer of male hormone. Histological differentiation of the gland epithelium occurs at 14 days of age, when the level of male hormone reaches the low threshold of the prostate for differentiation.

3. Seminal vesicle growth is slow in the early postnatal period, but a sudden and marked increase is evident, beginning at 26 days of age. This correlates with the ability of the organ to give a maximal response to stimulation at that period. Histological differentiation of the epithelium is found at 36 days of age, when the male hormone level reaches the high seminal vesicle threshold for differentiation.

4. The ovarian growth curve exhibits a fairly rapid rise, followed by a plateau

and a subsequent rise after 36 days of age. The initial growth followed by a plateau may be correlated with a marked increase in gonadotrophic potency of the pituitary, followed by a sudden drop in potency. There may also be a correlation between the gonadotrophic potency of the pituitary and ovarian differentiation.

5. The uterus grows fairly slowly until 36 days of age, when it undergoes very rapid growth. This is probably attributable both to an increased responsiveness of the organ and to an increase in female hormone level.

VII. Conclusions

The postnatal growth and differentiation of the reproductive system of the rat is under the influence of hormones to a marked degree. The gonads respond to gonadotrophin; the prostate to androgen from the ovary (maternal), the testis, and the adrenal; the seminal vesicles, to androgen from the ovary (maternal) and the testis; the uterus to estrogen from the ovary and the adrenal. The influence of adrenal androgen and estrogen has been demonstrated only in gonadectomized animals, and its significance in normal growth is not known.

The important factors which interact to influence the growth of the gonads and the accessory reproductive organs and to bring them to a functional condition are the levels of gonadotrophic hormone and gonad-secreted hormones and the threshold of sensitivity of the organs. These factors determine the specific type of growth (rapid or slow) and the age at which the functional state is reached.

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ORIGIN OF PIGMENT CELLS FROM THE NEURAL CREST IN THE MOUSE EMBRYO[1]

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IN A series of transplantation experiments, reported earlier (Rawles 1940*b*), in which the embryonic coelom of the White Leghorn fowl was used with considerable success as a site for testing the pigment-forming potency of various portions of mouse embryos of a potentially pigmented (black) strain, evidence was obtained which suggested that the migratory pigment-forming cells, the melanophores, take their origin from the neural crest, as in other vertebrates, e.g., amphibians and birds. The results of grafting a variety of embryonic mouse tissues, such as skin ectoderm plus the underlying mesoderm, somites with and without the adjacent neural tube, limb buds and prospective limb buds, from various body levels, showed that between 8½ and 12 days of gestation the capacity of the grafted embryonic tissue to produce melanophores spreads rather rapidly in both an anteroposterior and a mediolateral direction. It was found, for example, that grafts of skin ectoderm and mesoderm from somites of the anterior-trunk levels, including the level of the forelimb, of embryos of approximately 10 days gestation age, produced hairs fully pigmented (black), while similar grafts farther posteriorly, i.e., at the hind-limb level, produced only unpigmented (white) hairs. Later, by the 11th day, grafts from all body levels, including the posterior-limb level, produced hairs fully pigmented.

The fact that well-developed, structurally normal hairs entirely unpigmented could be obtained from a potentially pigmented (black) strain of mice, if certain body regions were grafted at a particular developmental stage, showed clearly that the ectoderm and its prospective derivatives have no inherent capacity for autonomous melanin-pigment formation. The pigment-producing cells must, therefore, come into the skin ectoderm and mesoderm from some outside source. That this source lies in, or is closely associated with, the neural tube was suggested by the fact that at certain stages in development when a graft from a particular axial level failed to produce pigmented hairs, e.g., the hind-limb region of a 31-somite embryo, the identical region would develop pigmented hairs if the implant was made to include the adjacent portion of the neural tube.

While the evidence from these experiments is, to say the least, extremely suggestive of the neural-crest origin of pigment cells, it was realized that further, and perhaps more precise, experiments were needed to confirm this mode of origin. The present report, therefore, is an account of the continuation of the preliminary experimentation with particular attention to correlating the morphological development of the neural crest with the observed spread in pigment-forming capacity.

The results presented in the following pages have been obtained from an examination of well over 200 coelomic

[1] All the illustrations were prepared by my husband, Mr. John Spurbeck. It is with deep appreciation that I acknowledge my indebtedness to him.

grafts from donors ranging in age from 4 somites to 12 days and would seem to leave no reasonable doubt that the pigment-forming cells—the melanophores—found in the skin of the mouse and in its derivative—the hair—originate from the neural crest.

Materials and Methods

The embryos used as donors came from matings within a highly inbred strain of homozygous black mice, C 57, obtained originally from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. A wide variety of developmental stages has been used, the stages ranging all the way from approximately 8 days of gestation (4 somites) to 12 days. Pregnancies were timed from the observance of vaginal plugs; but, in view of the wide variation in development of embryos among individual females and even within the litter of a single female, a more accurate check of the morphological age seemed necessary for this type of experimentation. Therefore, the morphological age of each donor used was determined by somite counts. It is relatively easy to make accurate somite counts on living embryos up until about 10½–11 days (40+ somites) if they are stripped of all membranes and if the body of the embryo is stained lightly with a vital dye, such as Nile blue sulphate. A simple but effective method for applying the dye is to touch the surface of the body of the embryo, lying in the saline solution, lightly with the smooth tip of a fine glass rod which has been coated with a 2 per cent agar solution and stained for 24 hours in a 0.5 per cent solution of Nile blue. When the embryos are too old for reliable somite counts, various other external morphological characteristics, such as the degree of pigmentation of the eye, the extent of differentiation of the limb buds, length of tail, etc., can be relied upon for rather accurate staging.

To avoid keeping the donor embryos in saline (Locke's solution) any longer than absolutely necessary, the female mouse was kept in a state of mild anesthesia with nembutal during the period of transplantation, which usually extended over several hours. By so doing, it was possible to remove one embryo at a time from the uterus without disturbing the other members of the litter. Usually there were many more embryos than could be used for transplantations at any one sitting, so the extras were always fixed in either Bouin's or Allen's B15 solution to be used as controls for histological study.

The preparation of the donors for transplantation was briefly as follows: The embryo with its membranes intact was transferred from the uterus to a Syracuse watch glass containing warm sterile Locke's solution and was placed on a small electric warming stage under a binocular dissecting microscope. The membranes were then carefully dissected away, the body of the embryo lightly stained with Nile blue sulphate, and the somites counted. With the aid of steel beading needles (Nos. 17–19) and a Bowman's iris knife, ground down to about half its original size, portions of the body were isolated at various levels along the anteroposterior and mediolateral axes. Each isolate was transplanted to the coelom of a White Leghorn chick embryo[2] of 60–65 hours incubation to test its pigment-forming capacity. The host eggs were incubated at the usual temperature, approximately 38° C. (for further technical details see Rawles, 1940*b*). The grafts were left usually until the host chick

[2] Some of the White Leghorn eggs used in these experiments were purchased from the United States Animal Husbandry Experiment Station, Beltsville, Maryland, through the courtesy of Dr. T. C. Byerly.

hatched (i.e., for 17–18 days), occasionally longer, from several days until a week after hatching. The hosts were then dissected to expose the graft site in the peritoneal cavity. A thorough examination was made with the binocular dissecting-microscope for pigment cells that might be located either in the graft itself or in the smooth, transparent peritoneum in the region of the graft. When pigment-cell development was obvious macroscopically, the grafts were not always sectioned, although the material was preserved so that it could be examined histologically if necessary. In cases where no pigment could be detected or where there was any doubt concerning pigment development, the grafts were regularly prepared for histological examination. Either Bouin's fluid or Allen's B15 solution was used as a fixative. Sections were cut at 8 μ and stained rather lighty with Delafield's haematoxylin. Grafts from hosts which had died at or during the hatching period were preserved in 10 per cent formalin. While they were not suitable for sectioning, they could be cleared in wintergreen oil and examined *in toto* for hairs and pigment.

THE GRAFTS

When the hosts were dissected, it was found that the transplanted mouse tissues had differentiated into a definite body or graft in 242 cases out of 314 examined. As a rule, the grafts were small, rounded, or oval-shaped bodies ranging from 2 or 3 mm. to approximately 1 cm. in the greatest dimension. They were well vascularized and usually rather firmly attached to the inner wall of the abdominal cavity in the region of the hind limbs—sometimes almost imbedded in the body wall. Occasionally, attachment was made entirely with the mesenteries of the gut rather than with the body wall. The free surface of the graft was nearly always connected with some portions of the viscera, such as the intestines, mesenteries, cloacal wall, sex ducts, etc., by strands of connective tissue containing small blood vessels. Along these strands, donor melanophores were frequently found. Often there were extensive adhesions of the graft with the intestinal walls and mesenteries. The graft surface was always enveloped with the smooth, transparent host peritoneum. Owing to the fact that the implanted tissue tended to round up into a ball or vesicle, skin and hairs, when present, were as a rule found directed toward the interior of the graft rather than growing directly into the body cavity.

Grafts always remained in the host coelom sufficiently long to insure pigment-cell development. In the youngest donors, those of approximately 8 days gestation, the total age of the grafted tissue after a minimum of 17 days in the host would be equivalent to that of a 5-day postnatal mouse. At birth, in this particular strain of mice, melanophores concentrated in the hair primordia and distributed lightly in the dermis of the skin between the hair primordia can be easily detected, with magnification, in a cleared whole mount of skin from any part of the dorsal surface of the body (see Fig. 9).

The melanophores produced by the grafted tissues are typical in every respect of those of the normal mouse (compare Figs. 10, 13, 16, with Figs. 9, 14, 15). Whether or not they would be produced by the grafted tissues was found to depend upon the age and axial level of the donor from which the implant was originally taken. Differences in these respects will be described in following experimental sections.

The Experiments

SERIES 1. DONOR EMBRYOS 4–23 SOMITES

In this group of experiments, portions of the body of the embryo were isolated at various axial levels in such a way as to test tissues of the central nervous system (C.N.S.) and adjacent tissues of the somite and lateral-plate regions, separately and in combination, for pigment-cell formation in coelomic grafts. Figure 1 illustrates the general method of isolation. The particular levels tested at any specific developmental period between 4 and 23 somites may be seen by referring to Table 1, second column. The isolates were taken from either side or from both sides. For example, if the neural tube was included with the somite and lateral plate of one side, the somites and lateral plate of the opposite side were also transplanted.

It will be seen from Figure 1 that the isolations fall into four types: Type A, transplants containing only neural tissue, i.e., undifferentiated brain or neural tube, depending upon the level. Usually the neural tissue at any particular level was taken in its entirety, but occasionally it was divided into right and left halves, and each half was grafted separately. (No attempt was made to remove the thin layer of overlying ectoderm prior to the 15-somite stage.) Type B, transplants consisting of somites and lateral plate—the overlying ectoderm and the underlying endoderm included. Type C, transplants consisting of the neural tube plus the adjacent somite and lateral-plate regions. Type D, transplants consisting of the entire region of the embryo posterior to the last-formed somites, i.e., the primordium of the neural tube, prospective somites, and lateral plate. This was sometimes divided into right and left halves, and each half was grafted separately.

RESULTS

The data pertaining to pigment-cell development obtained from this series of transplantations, in which 81 grafts were recovered, have been summarized in Table 1, the plus signs indicating the presence of pigment, the minus signs its absence. Inspection of the data will show that isolates of pure neural tissue, Type A, produced pigment in 14 out of 20 cases. The pigment was produced by specialized branched cells—melanophores—exhibiting the characteristics of those of the normal mouse (see Figs. 10, 13, 16). In this group of grafts the melanophores were not found within the body of the graft proper, which was composed of differentiated neural tissue only, but were found either in the host peritoneum covering the surface of the graft or, more often, in the peritoneum in the immediate neighborhood of the graft. Histological sections through the peritoneum show that the melanophores are located in the connective tissue beneath the mesothelium. In this group of grafts the melanophores appeared to show a definite inherent tendency to wander away from their origin in the graft proper.

Isolates which contained portions of the body lateral to the neural tube but which included no neural tissue, Type B, produced no melanophores, hence no pigment in all the 30 cases recovered. Since the skin ectoderm was left intact over the somites and lateral plate when the original isolation was made, the grafts developed skin with hairs normal in every respect except that they contained no pigment, i.e., were white instead of the typical black color characteristic of this strain of mice (see Figs. 19 and 20).

Isolates which included neural tube

TABLE 1

DONORS, 4–23 SOMITES: SUMMARY OF THE NUMBER OF GRAFTS RECOVERED FROM TRANSPLANTING VARIOUS PORTIONS OF THE EMBRYO AT DIFFERENT AXIAL LEVELS WITH REFERENCE TO THE CAPACITY TO FORM PIGMENT CELLS

Donor Age in Somites	Axial Level of Isolate	Type of Isolation							Total No. of Grafts
		A C.N.S.*		B Somite and Lateral Plate	C C.N.S.+Somite and Lateral Plate		D C.N.S.+Prospective Somite and Lateral Plate		
4.....	S 1–4†	1+	.....	4−	1+	.....	.....	.....	11
	Ant. to S 1	2+	.....	.....	.....	.....	.....	.....	
	Post. to S 4	.....	.....	.....	.....	.....	1+	2−	
5.....	S 1–5	.....	.....	.....	1+	.....	.....	.....	3
	Ant. to S 1	1+	.....	.....	.....	.....	.....	.....	
	Post. to S 5	.....	.....	.....	.....	.....	1+	.....	
7.....	S 1–3	.....	.....	.....	.....	1−	.....	.....	6
	S 1–7	.....	.....	1−	.....	.....	.....	.....	
	S 4–7	.....	.....	.....	.....	1−	.....	.....	
	Ant. to S 1	.....	1−	.....	.....	.....	.....	.....	
	Post. to S 7	.....	.....	.....	.....	.....	2+	.....	
10.....	S 2–5	.....	.....	.....	1+	.....	.....	.....	4
	S 5–10	1+	.....	1−	.....	1−	.....	.....	
12.....	S 1–6	.....	.....	.....	.....	1−	.....	.....	2
	S 6–12	1+	.....	.....	.....	.....	.....	.....	
13.....	Fore- and midbrain	1+	.....	.....	.....	.....	.....	.....	5
	Mid- and hind-brain	.....	1−	.....	.....	.....	.....	.....	
	S 1–5	.....	.....	1−	1+	.....	.....	.....	
	Post. to S 13	.....	.....	.....	.....	.....	.....	1−	
14.....	Midbrain	1+	.....	.....	.....	.....	.....	.....	5
	S 1–6	1+	1−	1−	.....	.....	.....	.....	
	Post. to S 14	.....	.....	.....	.....	.....	1+	.....	
15.....	S 1–5	.....	.....	1−	.....	.....	.....	.....	4
	S 6–12	.....	1−	1−	.....	.....	.....	.....	
	S 13–15	.....	.....	1−	.....	.....	.....	.....	
16.....	S 1–4	.....	1−	.....	.....	.....	.....	.....	8
	S 4–6	1+	.....	.....	.....	.....	.....	.....	
	S 10–15	.....	.....	2−	.....	.....	.....	.....	
	S 11–16	.....	.....	1−	1+	.....	.....	.....	
	Post. to S 16	.....	.....	.....	.....	.....	2+	.....	
17–18.....	S 9–12	.....	.....	.....	.....	1−	.....	.....	6
	S 14–18	.....	.....	1−	1+	1−	.....	.....	
	Post. to 17–18	1+	.....	1−‡	.....	.....	.....	.....	
20.....	S 6–11	1+	.....	2−	.....	.....	.....	.....	9
	S 10–20	.....	.....	1−	.....	1−	.....	.....	
	Post. to S 20	.....	.....	2−‡	.....	.....	2+	.....	
22.....	S 6–12	.....	.....	2−	2+	1−	.....	.....	11
	S 13–17	1+	.....	2−	1+	.....	.....	.....	
	Post. to S 22	.....	.....	2−‡	.....	.....	.....	.....	
23.....	S 6–13	1+	1−	2−	1+	.....	.....	.....	7
	S 15–23	.....	.....	1−	1+	.....	.....	.....	

* C.N.S. = Tissue of central nervous system.

† S = Somite. ‡ Prospective somite and lateral plate in original isolate.

along with the somite and lateral-plate material, Type C, developed melanophores in 11 cases, failed in 8. Histological examination of the negative cases, interestingly enough, revealed no trace of neural tissue. Although neural tissue had been included in the original implant, it had failed to survive and develop in the graft during its period of growth in the coelom. These negative cases, therefore, actually belong in the category with grafts of Type B, which, it will be recalled, contained no neural tissue and produced no melanophores. In the 11 positive grafts in which neural tissue persisted and underwent differentiation, melanophores were found both within the grafts and outside of the grafts in the surrounding host peritoneum. Those which developed within the grafts were found chiefly in the developing hairs; a few in the dermis of the skin, scattered here and there between the hair follicles; none in the epidermis proper. The pigmented hairs which developed in this group of grafts are similar in every way to those illustrated in Figures 17 and 18. The melanophores are confined to the epithelial portions of the developing hairs. In the dermal papillae they cannot be distinguished. Within the hair bulbs the melanophores are crowded together and their processes so entwined that it is difficult to distinguish one from another. However, the melanin granules which they contain are identical with those in the processes of the less crowded melanophores found in the dermis of the skin and in the host peritoneum. The distribution of melanophores in the mesenteries of the host surrounding a typical graft of this group obtained from a 13-somite donor is shown in Figure 11. In general, it was observed that grafts which contained numerous pigmented hairs showed little or no melanophore invasion of the host peritoneum, and vice versa. It would appear that the hair primordia somehow attract precursor melanophores to them, thus preventing their migration from the graft into the surrounding host tissues.

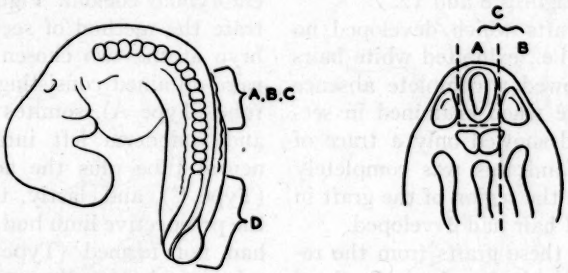

FIG. 1.—Diagram showing the four types of isolation made in the experiments of series 1. See Table 1 for the particular levels tested at a given developmental stage.

The final group of grafts of this series, Type D, listed in Table 1, in which the isolates contained neural tissue plus prospective somite and lateral-plate material, produced melanophores in 9 out of 12 cases. As in the preceding group of grafts, melanophores were found in the grafts concentrated in the developing hairs and invading the surrounding host peritoneum. The pigmented hairs shown in Figure 17 developed in one of these grafts obtained from a 7-somite donor. While the great majority of the hairs were as fully pigmented as those illustrated, some were occasionally found which contained very few melanophores

or none at all. Since these partially pigmented and pigment-free hairs were found more often in small isolated vesicles which had become more or less separated from the main body of the graft, it is very possible that the entrance of the precursor melanophores to these primordia had been wholly or partially blocked. In this group of grafts, as in the preceding, a correlation was observed between the hair content of the graft and melanophore migration into the surrounding host tissues. In several cases where hairs were comparatively few, melanophore migration into the host tissues was extensive—quite similar to that illustrated in Figures 8 and 12.

Of the 3 grafts which developed no pigment cells, i.e., exhibited white hairs entirely, 2 showed a complete absence of neural tissue when examined in section. The third showed only a trace of neural tissue, and this was completely separated from the region of the graft in which skin and hair had developed.

As a whole, these grafts from the region posterior to the last-formed pair of somites showed the best growth of any of the grafts of this series and produced the greatest quantity of melanophores per graft.

In summary, analysis of the foregoing data shows a definite correlation between melanophore development and the inclusion of neural tissue in the grafts. Of a total of 51 grafts in which tissue of the central nervous system was included in the original transplant (Types A, C, and D), 34 grafts ($66\frac{2}{3}$ per cent) developed normal pigment cells, while implants of somite and lateral plate exclusive of neural tube (Type B) produced no pigment cells in 30 cases (100 per cent). The fact, further, that structurally normal skin and white hairs could be obtained from a potentially black strain by excluding neural tissue precludes the possibility of pigment-cell origin from either epidermal or mesenchymal cells. From this series of experiments the conclusion is reached that the source of pigment either lies within, or is closely associated with, the tissues of the central nervous system.

SERIES 2. DONOR EMBRYOS 25–40 SOMITES

In this series of experiments, isolations were made at two selected levels, namely, the forelimb and hind-limb levels. As in the preceding series, the pigment-forming potencies were tested by transplantation to the White Leghorn chick embryonic coelom. Figure 2 will illustrate the method of sectioning the embryo at the two chosen levels. Isolates were obtained consisting of pure neural tube (Type A), somites (skin ectoderm and endoderm left intact) (Type B), neural tube plus the adjacent somites (Type C), and, lastly, the limb bud or the prospective limb bud, if the limb bud had not formed (Type E). Care was taken to isolate the neural tube quite free from other tissues, ectoderm and mesoderm. The ectoderm could be stripped off relatively easily. The separation of the mesenchyme was more difficult and laborious. However, it was found that a clean separation could be accomplished by gradually pushing the mesoderm away from the neural tube toward the somite with the fine edge of the iris needle-knife rather than cutting directly through from dorsal to ventral. Any loose cells adhering to the surface of the neural tube could be detected with suitable magnification. The final check, of course, was the histological examination of the resulting grafts which never showed skin and only rarely showed traces of cartilage. The great majority exhibited only differentiated neural tissue (spinal cord), typical of the portion

included in the transplant. During the course of the experimentation it was found that it was technically easier to transplant the fragile neural tube without injury if it was divided into narrow strips. In the majority of cases, therefore, the lateral half of the isolated neural tube was divided longitudinally into dorsal and ventral portions, and each was transplanted separately. But in some instances the entire neural tube or either lateral half was transplanted successfully.

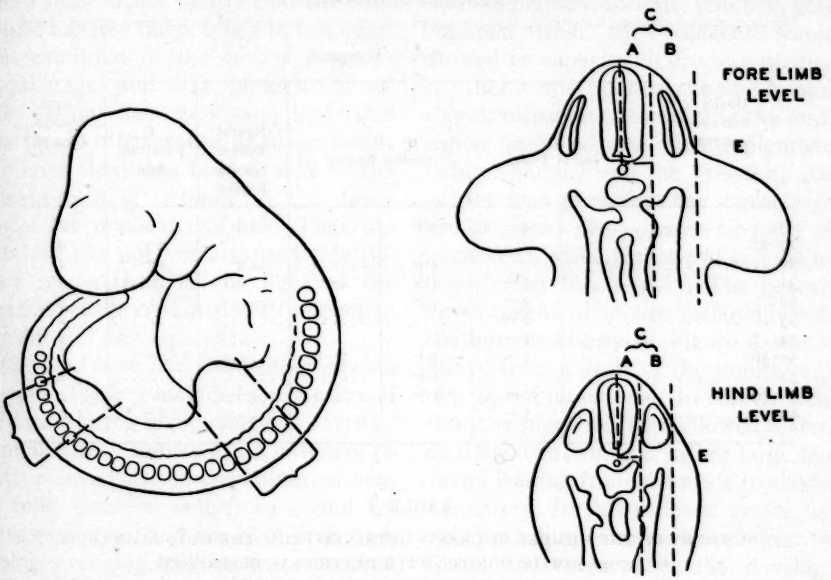

FIG. 2.—Diagram showing the four types of isolation made in the experiments of series 2. See Tables 2 and 3 for the various developmental stages used.

RESULTS

A total of 112 grafts was obtained from 153 cases in which the hosts remained alive until the hatching period or shortly before. The data relating to melanophore development have been summarized in Tables 2 and 3. The plus signs indicate the presence of pigment cells, the minus signs, their absence.

The forelimb level (somites 6 or 7–12 or 13).—Reference to Table 2 will show 46 grafts obtained from isolations made at the forelimb level of donors ranging from 25 to 40 somites. Of the 22 grafts recovered from transplants of various portions of the neural tube, only 1 produced pigment. In this exceptional case the entire segment of the tube was transplanted, but, according to the protocol, the isolation was not too clean—some mesenchyme cells were adhering—which, no doubt, accounts for the unusual result. The pigment cells which developed were found outside the graft in the peritoneum of the host, as in Type A grafts of the preceding series. In 5 cases grafts were recovered from both the dorsal and the ventral portions of the neural tube of the same donor.

Isolates of the somite region adjacent to the neural tube but not including any part of it developed pigment cells in 12 out of the 14 grafts recovered. In most of these grafts the melanophores were found concentrated in the developing

hairs (see Figs. 17 and 18). No very extensive migration into the host tissues was noted.

Somites and neural tube grafted together (Type C) produced positive results like those obtained from grafting somites (Type B). Since the neural tube alone produced no pigment in 21 out of 22 grafts, it is relatively certain that the source of pigment in these grafts lies within the adjacent somite region.

Isolated forelimb buds developed no

TABLE 2

SUMMARY OF THE NUMBER OF GRAFTS OBTAINED FROM THE FORELIMB LEVEL OF 25–40-SOMITE DONORS, WITH PARTICULAR REFERENCE TO THEIR PIGMENT-FORMING POTENCY

Donor Age in Somites	Type of Isolation						Total No. of Grafts
	A Neural Tube		B Somite Region		C Neural Tube+ Somite Region	E Forelimb Bud	
25		4−	3+		1+		8
27–28						4−	4
30	1+		1+		1+	1−	4
31		5−	1+	1−			7
32		2−	1+				3
33		2−					2
34		1−					1
35–36		2−	4+	1−		1−	8
37			1+				1
38–39		2−	1+		1+		4
40		3−				1−	4

TABLE 3

SUMMARY OF THE NUMBER OF GRAFTS OBTAINED FROM THE HIND-LIMB LEVEL OF 25–40-SOMITE DONORS, WITH PARTICULAR REFERENCE TO THEIR PIGMENT-FORMING POTENCY

Donor Age in Somites	Type of Isolation						Total No. of Grafts
	A Neural Tube		B Somite Region		C Neural Tube+ Somite Region	E Hind-Limb Bud	
25	1+	1−		3−	1+		6
26				2−	2+		4
28				2−	2+	2−	6
30				1−	1+	1−	3
31	1+	2−		2−	2+	1−	8
32	2+			2−			4
34		1−		1−			2
35–36	2+		1+			2−	5
37			1+	1−		3−	5
38–39		2−	4+	2−		4−	12
40		7−	1+		1+	2−	11

pigment in the 7 cases examined, yet structurally normal skin and white hairs were found in every case. If the adjacent somite region was included with the limb bud, as was done in a few cases, not listed in the table, pigmented hairs developed. This result is to be expected, since the somite region alone at these stages produced pigment regularly.

In summary, the transplants just described show rather clearly that the neural tube has lost the potency to form pigment exhibited in the earlier developmental stages and that the adjacent somite region has now acquired this capacity. In other words, at the anterior-limb level there has been a shift of the pigment-forming potency in the direction of the mediolateral axis. That the limb bud has not yet acquired this potency seems clear in the light of the negative results obtained with respect to pigment-cell development.

The hind-limb level (including somites 24–29).—Table 3 includes a summary of the data relating to pigment-cell development obtained from an examination of 66 grafts recovered from transplanting neural tube, somites, neural tube and somites combined, and the hind-limb bud, at stages ranging from 24 to 40 somites. (It is to be understood that these regions when isolated from 25–26-somite embryos would consist of prospective somites and limb bud.)

It will be seen from Table 3 that grafts of pure neural tube (Type A) produced melanophores up until the 35–36-somite stages; after which, only negative results as regards pigment-cell development were obtained. Figure 12 represents a neural-tube graft from a 32-somite embryo and shows the rather extensive migration of melanophores into the host mesentery which often occurs in such cases. While no grafts were recovered from pure neural transplants at 26-, 28-, and 30-somite stages, the fact that, at these stages, grafts of somite material including neural tube (Type C) produced pigment, while the same grafts without neural tube (Type B) gave no pigment at all, is convincing evidence that the neural tissue was the source of the melanophores that differentiated in these grafts.

At the stages when the neural tube exhibits pigment-forming potency, grafts obtained from the adjacent somites showed no capacity to produce pigment. In other words, up until the 35–36-somite stages, transplants containing the somite region produced skin with unpigmented (white) hairs. From the 37-somite stage on, the data show that the somite region has acquired the potency to form pigment cells, while the neural tube is now negative in this respect. The beautiful development of mouse melanophores in the gut mesentery of Figure 8 was obtained from a graft of the somite region of a 39-somite embryo. In this particular case the pigment cells followed a strand of tissue containing a rather large blood vessel leading from the graft to the host mesentery. In this series of grafts, as in the preceding, the melanophores were found concentrated in the developing hairs and in the peritoneum of the host when hairs were few or absent entirely.

In summary, this group of transplants has demonstrated that, before the appearance of the hind-limb bud and during its earliest development, the source of the melanophores lies within the neural tissue destined to form spinal cord. Later, as development proceeds and the limb bud becomes a rather definite structure, the pigment source shifts laterally along the mediolateral axis from the neural tube to the adjacent somite region. The limb bud itself showed no evidence of having yet acquired pigment-forming potency, since unpigmented (white) hairs

developed in all the 15 cases examined (see Figs. 19 and 20).

SERIES 3. DONOR, 11–12 DAYS

In this group of transplantations dealing with later developmental stages when the embryos had increased greatly in size, the method of isolation was, of necessity, changed somewhat. Rather than attempting to graft regions of the body, as heretofore, areas of skin ectoderm plus the underlying mesoderm, not exceeding 1 square millimeter, were now removed from the posterior lateral head region, from the prospective shoulder and rump, and from the upper surface of the proximal portion of each limb bud and grafted to the coelom, as usual. A total of 38 grafts was obtained.

RESULTS

Grafts from the head and trunk regions of 11-day embryos produced pigment cells in all the cases examined (10), while grafts from the limb buds showed no trace of pigment in 13 cases, although skin and hairs developed regularly. The pigment-free hairs which developed in these grafts are like those obtained from donors of earlier developmental stages and illustrated in Figures 19 and 20. By the 12th day the pigment-forming potency has spread along the mediolateral axis sufficiently far to include the limb buds, as evidenced by the fact that skin ectoderm and mesoderm grafted from the limb buds (6 cases) at this time produced pigmented hairs, like similar grafts from the head and trunk regions. It is concluded, therefore, that by the 12th day precursor melanophores have reached their definitive positions in the primordium of the skin of the body surface in general.

The melanophores which developed in these grafts are like those of previous series illustrated in Figures 13–16. Hair development was somewhat more abundant than in the preceding two series of grafts, and, since the total age of the grafted tissue was approximately equal to 10 days after birth, most of the hairs were longer, reaching several millimeters. They appeared to be as completely and as intensely pigmented as those of a normal 10-day mouse. Very few melanophores developed outside the grafted tissues in the host peritoneum. Of the 25 grafts of this series which produced pigment cells, only 11 showed even a trace of melanophore migration into the surrounding host tissues.

Relation between the Appearance of Melanophores in Grafts and the Development of the Neural Crest

Before proceeding further with an analysis of the data, let us turn our attention to the development of the neural crest in an attempt to determine whether or not the mediolateral spread in pigment-forming capacity, observed first in the anterior regions and gradually later in more posterior levels as the embryo continues its development, can be correlated with the morphological development of the neural crest.

In the mouse embryo the development of the neural crest follows closely the pattern described by Holmdahl (1928) for four other mammalian embryos: the cat, the rabbit, the roe, and a European ground squirrel (*Spermophilus citillus*). It appears, first, in the midbrain region, before the closure of the neural folds and shortly after the formation of the fourth pair of somites. From here it appears more and more caudally as the embryo continues to differentiate in that direction. In the segmented body region, where crest development is somewhat more regular than in the cranial (unsegmented) region, its development is typi-

cally as follows: cells of the dorsal portion of the closed neural tube, just beneath the thin surface layer of ectoderm, appear to undergo some rearrangement and proliferation, forming a slightly thickened mass of rather loosely joined cells (see Fig. 7). From this mass, cells begin to wander out on each side in two directions: (1) laterally, toward the dorsal portion of the somite, pushing between it and the overlying ectoderm, and (2) ventrally, along the sides of the neural tube, between it and the laterallying somite. While the great majority of crest cells appear to take the ventral route, it is not uncommon in transverse sections to find the two strands in the forked configuration shown in Figure 6. In the meantime, while the crest cells are continuing to stream out from the dorsolateral region of the neural tube in increasing numbers, the somite, which at the beginning of crest formation is seen in its primary developmental stage, begins to differentiate into dermatome, myotome, and sclerotome. When the migrating crest cells reach the loose mesenchyme cells of the developing sclerotome, they intermingle and cannot be distinguished. By the time the somite has attained the stage of differentiation illustrated in Figure 3, the dorsal boundaries of the neural tube have become sharply defined, and crest cells can no longer be found migrating from it. The region between the neural tube and the somites is, by now, filled with mesenchyme cells contributed by both the crest and the somite, but histologically indistinguishable. Owing to its characteristic progressive development and the fact that at any given level the crest completes its development rapidly and ceases to exist as a definite morphological structure, it is not found as a continuous body extending throughout the length of the embryo.

After the development of approximately 20 pairs of somites, all these stages in the development of the crest may be found in the trunk region of the same embryo by examining different axial levels in transverse sections. In the posteriormost regions, where segmentation of the mesoderm into somites is going on, the crest will be found in the beginning phase illustrated in Figure 7; more anteriorly the crest will be found in its migratory phase, with cells streaming out laterally and ventrally, as illustrated in Figures 4, 5, and 6; and, finally, in the anteriormost regions the crest will have left the neural tube entirely and reached the stage of differentiation illustrated in Figure 3. It can be seen, then, that a segment of neural tube isolated from a level at which the crest has not begun its migration would contain the entire prospective crest, while a similar segment isolated from a level at which the crest has completed its migration would contain no crest cells whatsoever. Likewise, portions of the embryo lateral to the neural tube, such as somites, isolated before the crest cells had entered their migratory phase, would be crest-free, while the same type of graft isolated after the crest had migrated into it would contain numerous crest cells. By allowing such isolates to continue their growth in a site favorable to pigment-cell development, e.g., the embryonic chick coelom, it is possible to determine whether or not there is any relationship between the appearance of pigment cells in the graft and the phase of development of the crest at the time the isolation was made.

Examination of the data presented in the preceding section shows that there is a very definite correlation between the development of the crest and the appearance of melanophores in the grafts. For example, in the first series of experiments dealing with embryos of early somite

stages (4–23 somites), the development of melanophores in the grafts was found to be dependent upon the inclusion of neural tissue. In no case did isolates of somites and lateral plate produce pigment cells. Histological examination of control embryos, at the particular levels tested, reveals that the crest was either confined entirely to the neural tube or had not migrated sufficiently far from it to be included in more lateral regions. Transplants of the somite and lateral-plate regions would therefore be crest-free. Similarly, crest development and the production of melanophores by the grafted tissue can be correlated in the second series of experiments, in which donors of later somite stages were used. The data presented in Table 2 show that, by the 25-somite stage, the neural tube at the forelimb level has lost the potency to form pigment cells exhibited at earlier stages and that the adjacent somite region has now acquired this capacity. At the hind-limb level at this same stage, where the somites are just now in the process of being cut off from the unsegmented lateral mesoderm, the results relative to pigment-cell formation in the grafts show the reverse condition, i.e., the pigment-forming capacity is confined to the neural tube. If we compare transverse sections of the two levels in control embryos, we find a noticeable difference in the development of the crest. At the forelimb level the crest has reached the stage of differentiation illustrated in Figure 3, i.e., it has grown out from the neural tube and intermingled with the mesenchyme of the developing somite. Grafts of the somite region, therefore, would contain numerous crest cells; grafts of neural tube would be crest-free. At the hind-limb level, the youngest portion of the embryo, the crest is found in the early developmental phase illustrated in Figure 7, i.e., confined to the dorsal portion of the neural tube. A graft of neural tube at this stage would therefore contain the crest; a graft of the somite region would not.

The lateral spread in pigment-forming potency observed at the forelimb level from the 25-somite stage on is not apparent at the hind-limb level until the development of approximately 37 somites. Sections through the hind-limb level of a 37-somite embryo show that the crest has now reached a stage of development comparable to that of the forelimb level at the 25-somite stage. Thus, as long as the crest can be recognized in sections as a definite morphological structure, it can be clearly demonstrated that pigment-cell formation in the grafts is dependent upon the inclusion of crest cells. Such clear-cut correlation between pigment-cell formation in grafts and neural-crest development constitutes proof that the prospective pigment cells of the mouse embryo take their origin from the neural crest.

In birds these results find a close parallel in the results of the intracoelomic transplantation experiments of Ris (1941), in which he, also, correlated the development of pigment cells (melanophores) in grafts with the morphological appearance of the neural crest at the time of isolation. By transplanting portions of Barred Plymouth Rock embryos (and other birds) from various axial levels at successive stages in their development to the embryonic coelom of White Leghorns, he was able to show that only those isolates containing the neural crest were capable of producing melanophores in grafts. Comparison of the grafts of mouse tissue with those obtained by Ris reveals striking similarity in pigment-forming potency and pigment-cell development in these two classes of vertebrate embryos.

Having, then, furnished proof that the

prospective pigment cells of the mouse originate only from the embryonic neural crest, it is now possible to locate prospective crest cells, in stages prior to crest formation, by testing the capacity of a given embryonic area for pigment-cell production in grafts. Reference to the data (Table 1) will show, for example, that at the 4-somite stage, when there is no visible sign of crest formation, pigment cells can be obtained from isolates at any level if they include the primordium of the neural tube—the region from which the crest will arise later. Somite and lateral-plate grafts alone showed no pigment-forming capacity. If, however, the neural-tube primordium is included with the somite and lateral-plate material, the transplants will now develop pigment cells. The same conditions hold in the transplants of the embryonic region posterior to the last somite (Type D, Table 1). In these posteriormost levels the crest has not yet arisen, yet pigment cells are produced in quantity whenever the isolate contains the neural-tube primordium or recently formed neural tube. Prospective somite material alone, i.e., the unsegmented mesoderm (and ectoderm) lateral to the neural tissue, exhibits no pigment-forming potency.

Again the results find a close parallel in the experimentation with chick embryos. Ris (1941) demonstrated that the entire neural-tube primordium during the process of closure was capable of producing pigment cells. Immediately after closure this capacity was restricted to the dorsal portion only (prospective crest). Using *in vitro* culture methods, Dorris (1938) was able to obtain pigment cells from explants of the dorsal neural folds (neural-crest region) of early somite embryos. Eastlick (1940) and Rawles (1940*a*) traced the pigment-forming potency still further back in development to presomite blastoderms, finding that the area capable of producing pigment cells coincided with the area known to form neural tissue in chorio-allantoic grafts (Rawles, 1936). The relatively wide distribution of the pigment-forming potency of the presomite blastoderm becomes restricted to the neural tube in early somite stages and, finally, to the neural crest itself, when it becomes segregated into a definite primordium.

After the crest has completed its outgrowth from the neural tube and become histologically indistinguishable from ordinary mesenchyme, its lateral migration can be followed experimentally by testing the capacity of a given area of embryonic tissue for pigment-cell production in grafts. By so doing, it has been possible to follow the prospective pigment cells (melanoblasts) to their definitive positions in regions most distant from their source, e.g., into the limb buds. The data show that, by the time an embryo has developed 40 pairs of somites (approximately 10½ days), skin ectoderm and adhering mesoderm removed from the head and from any level of the trunk will produce pigmented hairs in grafts. But limb buds isolated at this time, and prior to this time, invariably produce hairs completely free of pigment cells. Only by including the adjacent somite region with the limb primordium can pigmented hairs be obtained from grafted limb buds at or prior to this time (cf. Eastlick, 1939). Later on, between the 11th and 12th days, precursor pigment cells do make their way into the limb buds, as evidenced by the fact that grafts of skin ectoderm and adhering mesoderm from the limb buds at this time develop pigmented hairs. From these data it is concluded that, by the 12th day, prospective pigment cells have migrated from their origin in the neural crest to their definitive positions

in the skin and hair primordia of the body surface in general.

In birds (fowl) the migration of melanoblasts from their origin in the neural crest to their definitive locations in the skin and feather germs has been followed by various transplantation experiments. Willier and Rawles (1940) showed that prospective pigment cells are present in head and trunk ectoderm and mesoderm by 62 hours incubation but are not present in the wing bud until approximately 80 hours or in the leg bud until 96–100 hours (cf. Watterson, 1938; Eastlick, 1939; Ris, 1941). The technique employed by Willier and Rawles (1940) and Rawles (1939), whereby neural-crest cells (prospective melanophores) of one breed can be transplanted to the wing bud of another breed, has furnished the most conclusive evidence for the migration of prospective pigment cells into feather germs.

In amphibians (urodeles) a variety of transplantation, explantation, and deficiency experiments have shown that the pigment cells originate in the neural crest (see the review by DuShane, 1943). Recently Lopashov (1944), working with embryos of three species of teleosts has presented evidence for pigment-cell derivation from the "anlage of the nervous system" (presumably, the neural-crest region). Interestingly enough, as DuShane (*loc. cit.*) points out, the earliest statement recorded relative to a definite relationship between pigment cells and the neural crest was made by Borcea, more than forty years ago, on the basis of a study of several species of bony-fish embryos. The chief group of vertebrate animals now remaining in which there is no experimental evidence available for linking the origin of pigment cells with the neural crest is the reptiles. On comparative grounds alone it would be surprising if they, too, did not conform. Branched, migratory, melanin-producing cells of striking morphological similarity have been observed in the epidermis and in many mesodermal structures of all the classes of vertebrates. Their structure, behavior, and origin suggest strongly that they are homologous cells. If we may generalize from the conditions prevailing in teleosts, urodeles, fowl, and the mouse, the conclusion appears inevitable that in the vertebrates, in general, melanin pigmentation in its great variety of forms is produced by one type of cell, the melanophore, which originates in the neural crest and migrates into the various pigmented portions of the body, particularly the skin and its derivatives.

Dependence of Hair Pigmentation upon Pigment Cells Derived from the Neural Crest

It is generally assumed on the basis of histological studies and the Dopa reaction that the epidermal cells of mammals are able to produce melanin pigment independent of the branched pigment cells, the melanophores (see the extensive review of Biedermann, 1928). The results of the present experiments demonstrate the invalidity of this assumption in one mammal, the mouse. Skin and hairs, normal in every respect except for the complete absence of pigment and pigment cells, have been obtained regularly, throughout the series of grafts, whenever neural crest, the source of the pigment cells, has been excluded from the isolate (see Figs. 19 and 20). Such results prove conclusively that melanin pigmentation of the epidermis and its derivative, the hair, is not autonomous but is entirely dependent upon one particular type of cell, the melanophore. Furthermore, since hair can develop normally in the complete absence of melanophores and

since melanophores can develop normally outside the hair primordia even in the peritoneum of the chick, it is quite evident that pigment-cell development and hair development can go on separately and independently.

The identical condition exists in birds where advantage has been taken of the excellent opportunity thus afforded for analyzing the roles of the two components—the melanophore and the feather germ—in the formation of color pattern. By introducing prospective melanophores (melanoblasts) from one breed or species into the developing feather germs of another, which possesses a different plumage color and pattern, it has been possible to demonstrate that the color and pattern of the resulting feather are invariably like that of the individual which furnished the melanophores (Willier and Rawles, 1940; Rawles, 1939, 1944, 1945). Moreover, microscopic examination of the resulting feather revealed the interesting fact that the individual melanophores, without exception, had deposited in the foreign feather cells melanin granules characteristic of their own particular species. Such consistent results from extensive experimentation with embryos of many breeds of common fowl and other birds, using several transplantation methods, have led to the conclusion that the melanophores control the color and the basic color-pattern of a feather and the feather germ its morphogenesis. In the absence of melanophores a structurally normal feather can be formed, but it cannot become pigmented. For a discussion of the relationships between the two components and their interaction in the production of the variations of plumage pattern found in different feathers of the same individual see Willier (1942, 1947).

Having, then, clearly demonstrated that melanophores are essential for the pigmentation of hair, just as they are essential for the pigmentation of feathers, the possibility of introducing prospective melanophores from one pigmented strain of mice into the developing hairs of mice of different strains becomes very real. A combination of the intra-coelomic–grafting and the skin-grafting techniques as employed by Rawles (1944) would seem most suitable. In fact, the first step, that of producing an area of pigment-free skin and hairs from a potentially pigmented mouse, has already been accomplished. Only the second step remains—that of retransplanting the area of pigment-free skin from the intra-coelomic graft to a newly born mouse. The results of the skin-transplantation experiments of Reed and Henderson (1940) with newly born mice encourage one to believe that prospective pigment cells will migrate from the surrounding skin into the pigment-free hairs of the graft (cf. Rawles, 1944).

During the course of the present investigation, certain observations have been made which would seem to indicate that the developing hair, like the developing feather, exerts considerable influence on the distribution and subsequent development of the prospective melanophores. For example, it was found that practically all the melanophores which developed in the grafts are located in the developing hairs. Comparatively few are found in the dermis and none to speak of in the epidermis. It was also found that melanophores could, and often did, develop independently, i.e., outside the graft entirely, in the peritoneum of the chick host. Whether or not, however, they developed in the host tissues depended to a large extent upon the hair content of the graft. If hairs were numerous, few or no melanophores were found outside the graft; if hairs were absent, as, for example, in grafts

of early neural tube (Table 1), all the melanoblasts produced by the isolate developed outside, in the host peritoneum (see Fig. 12). Such observations lead one to assume that the developing hairs exert an attractive force of some kind upon the prospective pigment cells, causing them to enter the hair primordia. Certainly, when hairs are available, melanoblasts find their way into them.

Evidence of a similar correlation between hair development and melanophore development is, interestingly enough, found in the normal mouse. Examination of skin from the hair-covered regions of the body shows melanophores concentrated in the hair bulbs; a few scattered here and there in the dermis between the hair follicles; and none at all in the epidermis. But skin from regions of the body where no hair develops or where hair development is greatly reduced, such as the nose, ears, soles of the feet, tail, etc., presents quite a different situation. In these regions melanophores are found, in relatively large numbers, in the dermis and in the basal layer of the epidermis. Steiner-Wourlisch (1925) in her study of the pigmentary system of the grey house mouse obtained a positive Dopa reaction from the epidermis and the dermis of these same regions. Elsewhere, with the exception of the hair bulbs, which always react positively, the reaction to Dopa was negative.

That the prospective melanophores arising in the neural crest eventually make their way into the developing hairs and bring about their pigmentation is definitely established from the observations recorded in the present work. It remains now to follow the details of the pigmentation process, i.e., to determine when and under what physiological conditions the pigment cells penetrate the epidermis of the hair primordium and how they actually release their pigment granules to the hair cells. Studies similar to those carried out by Watterson (1942) and Foulks (1943) on developing feathers should prove most informative.

Summary

1. The pigment-forming potency of various portions of mouse embryos of a homozygous black strain (C 57), ranging in age from approximately 8 to 12 days gestation, was tested by isolation and transplantation to the coelom of White Leghorn embryos of 60–65 hours incubation. In this favorable site the transplants were allowed to grow until the host hatched, i.e., for approximately 18 days.

2. Isolations were made at various axial levels in such a way as to test tissues of the central nervous system, adjacent tissues of the somite and lateral plate, and limb-bud regions, separately and in combination for pigment-cell production in the grafts.

3. A mediolateral spread in the pigment-forming capacity of the various portions isolated was observed, first, at the anterior levels and gradually later at more posterior levels as the embryo continued to differentiate along its anteroposterior axis.

4. A definite correlation was found between the presence of pigment cells in the graft and the phase of development of the neural crest at the time the isolation was made.

5. The data demonstrate clearly that only those tissues containing presumptive neural crest, histologically recognizable neural crest, or cells migrating from the neural crest can produce melanophores.

6. Skin and hairs, normal in every respect except for the complete absence of pigment and pigment cells, have been ob-

tained regularly throughout the series of grafts, whenever neural crest, the source of the pigment cells, has been excluded.

7. The development of structurally normal white hairs from a potentially pigmented mouse is conclusive proof that the formation of melanin pigment in the ectoderm and its derivative, the hair, is not autonomous but is entirely dependent upon one particular type of branched, pigment-forming cell—the melanophore—which originates in the neural crest.

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PLATE I

Figs. 3–7.—A series of photomicrographs of transverse sections through different levels along the anteroposterior axis of a normal mouse embryo of 27 somites, showing successive stages in the development of the neural crest. The embryo was fixed in Allen's B15, stained with Heidenhain's iron haematoxylin, and sectioned at 8 μ. ×210.

FIG. 3.—Level of 10th somite. Neural crest has completed its migration from the neural tube. Dorsolateral boundaries of neural tube now sharply defined. Note crest cells histologically indistinguishable from cells of sclerotome.

FIG. 4.—Level of 19th somite. Crest cells are being proliferated from neural tube and can be followed laterally and ventrally in section.

FIG. 5.—Level of 21st somite. Crest in earlier developmental phase than shown in preceding figure. Fewer cells have migrated from neural tube—dorsal portion still relatively thick. Note strand of crest cells between neural tube and somite.

FIG. 6.—Level of 23d somite. Early phase in crest development. Note the characteristic forked configuration of the two strands of migrating crest cells (see p. 259).

FIG. 7.—Level of 27th somite. Early crest formation. Note accumulation of cells in dorsal portion of neural tube. Crest cells just beginning to wander out from origin in dorsal mass.

PLATE II

FIG. 8.—Portion of mesentery of a 20-day White Leghorn host embryo showing numerous mouse melanophores. Obtained from grafting the somite region at the right-hind-leg level of a 39-somite mouse embryo. Mouse melanophores migrated into mesentery via a strand of tissue containing a blood vessel. ×15.

FIG. 9.—Unstained, cleared, whole mount of skin from shoulder region of a newly born normal mouse of donor strain (C 57). Note melanophores (*M*) in dermis, scattered lightly between hair primordia (*H*). ×165.

FIG. 10.—Unstained, cleared, whole mount of portion of peritoneum of White Leghorn chick host at hatching, showing migration of typical mouse melanophores from near-by graft of somite and lateral-plate material isolated at the forelimb level of a 25-somite donor mouse embryo. Note melanophores like those in normal skin of Fig. 9. ×165.

FIG. 11.—Intra-coelomic graft (*G*) obtained from an isolate of neural tube plus the adjacent somite and lateral-plate regions of a 13-somite mouse embryo at level of somites 1–5. Note three patches of melanophores developing in surrounding host mesentery. ×15.

FIG. 12.—Intra-coelomic graft (*G*) obtained from an isolate of the dorsal half of the neural tube of a 32-somite mouse embryo at the level of the hind-limb bud. Note the migration of melanophores from the graft into the mesentery of the host chick. ×15.

FIGS. 13 and 16.—Typical mouse melanophores developing in the White Leghorn host peritoneum in region of graft. Same case as Fig. 10, enlarged. Compare with melanophores developing in normal skin, shown in Figs. 14 and 15. ×450.

FIGS. 14 and 15.—Typical melanophores from the skin (dermis) of a normal newly born mouse. Same as Fig. 9, enlarged. Note that melanophores are of same type as those of Figs. 13 and 16. ×450.

PLATE III

FIG. 17.—Section through pigmented (black) hairs developing in a coelomic graft. Obtained from a 7-somite mouse embryo by isolating the entire region posterior to the 7th pair of somites. ×450.

FIG. 18.—Cleared, unstained whole mount of a portion of a graft in which numerous pigmented (black) hairs developed. Obtained from an isolate of skin ectoderm and underlying mesoderm from the somites at the right forelimb level of an 11-day mouse embryo. ×100.

FIG. 19.—Section through pigment-free (white) hairs developing in a graft of somite and lateral-plate material (plus overlying ectoderm) from the 24–25-somite level of a 25-somite mouse embryo. Note the normal development of the hair in the complete absence of pigment cells. ×450.

FIG. 20.—Cleared, unstained, whole mount of several pigment-free (white) hairs obtained from a graft of the hind-limb bud of a 40-somite mouse embryo, grown for 18 days in the White Leghorn coelom. Note hairs normal in every respect except for the complete absence of pigment. ×150.

PLATE I

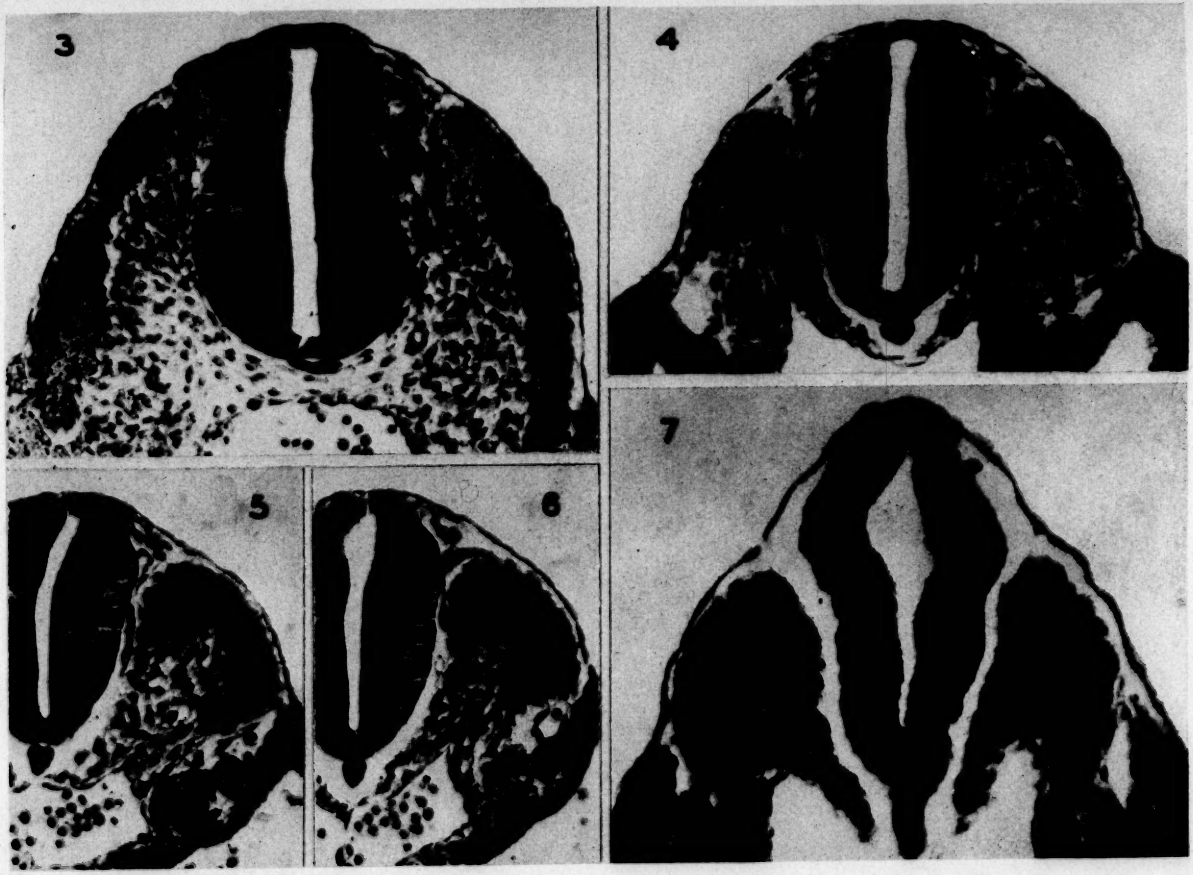


PLATE II

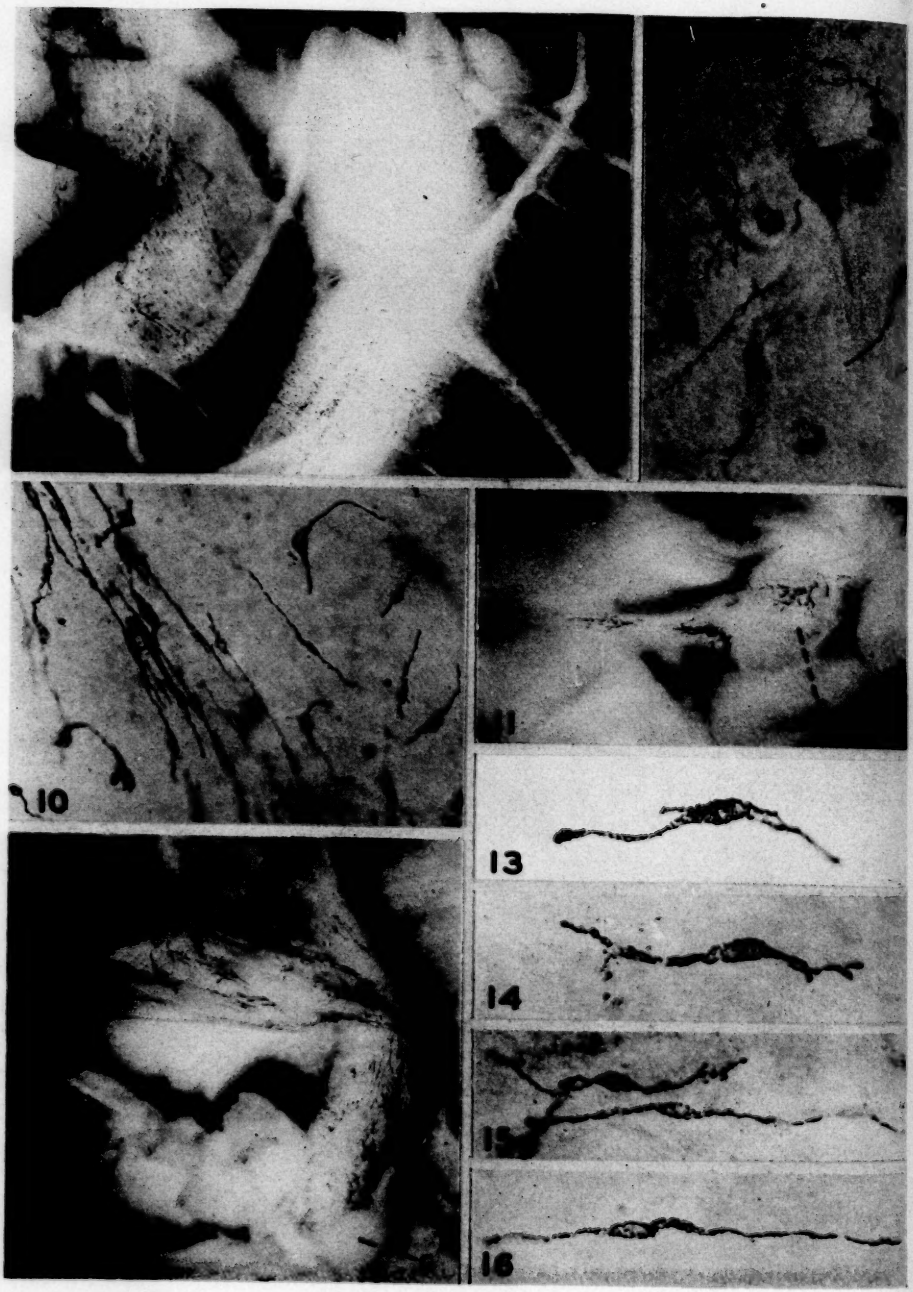
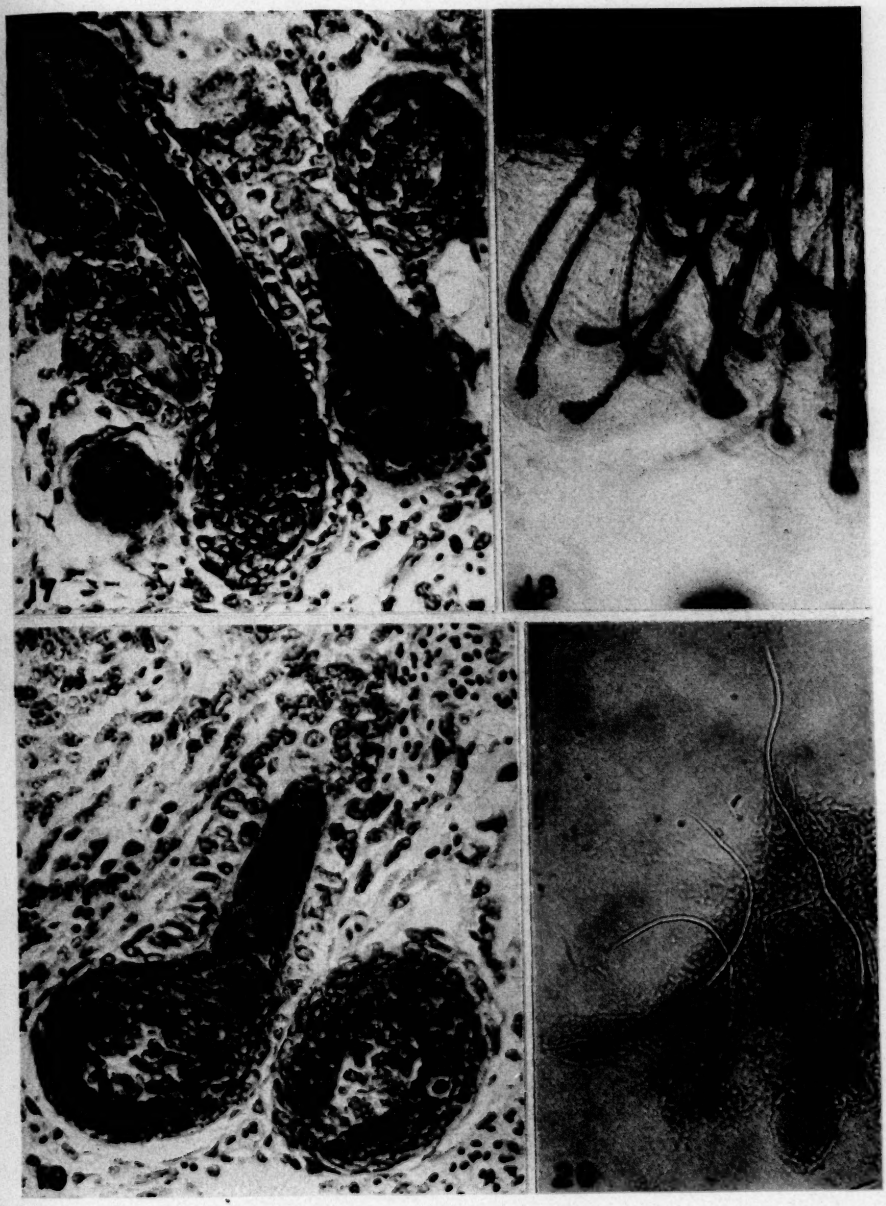


PLATE III



UNIV. OF MICH.

SOME RELATIONS BETWEEN TERRITORY, SOCIAL HIERARCHY, AND LEADERSHIP IN THE GREEN SUNFISH (LEPOMIS CYANELLUS)[1]

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THERE is a basic and widespread tendency for living things to aggregate, more often than not with mutual benefit (Allee, 1931). Integration of vertebrate aggregates is influenced by three major principles of behavior—territoriality, hierarchy, and leadership. Many species appear to organize in accordance with but one of these principles; nevertheless, in most instances traces of the operation of all three may eventually be recognized.

The modern concept of territory was first presented by Altum (1868) and Howard (1907–14). Although many observations have been gathered since Howard's (1920) stimulating book, few detailed studies of territorial behavior are yet available. Noble (1939*a*) gives a simple, inclusive definition of territory as "any defended area." Nice (1941) reviews the literature on birds and classifies a number of types of territory with regard to mating, nesting, feeding of young, roosting, etc. Territorial behavior seems to be most varied in birds but is also prevalent in the bony fishes, reptiles, and mammals.

Social hierarchies in domestic fowl were described by Schjelderup-Ebbe (1922, 1935). Small flocks of hens maintain a linear system of dominance, which has come to be called the "peck-right." The dominant bird has the right to peck all the others without, in turn, being pecked; the next in rank can attack all except the first; and so on down the line. Masure and Allee (1934*a*, *b*) confirmed the existence of this social order in chickens but found that certain other species of birds have a more flexible type of relation, which they named "peck-dominance." The dominant individual delivers more blows than it receives, but the subordinate often retaliates, and the outcome of any single contact is not strictly predictable. Masure and Allee observed that certain members of a group are more aggressive in one place than in another, and Allee (1942) suggests that this territoriality may be an underlying factor in the peck-dominance type of society.

Still other kinds of relations between territory and hierarchy have been proposed by Diebschlag (1941) for pigeons and by Greenberg and Noble (1939, 1942, 1944) for the American chameleon, *Anolis*. Diebschlag discovered that pigeons defend both residence points of limited area and "influence spheres," which may extend to an entire laboratory cage. Individuals were observed to engage in peck-dominance exchanges with others that held residence areas within the formers' spheres of influence. Greenberg and Noble recorded similar situations in *Anolis* lizard groups; however, the relations between the territory-holding males were of the peck-right type.

Leadership-followership phenomena

[1] This study originated from a suggestion by Dr. W. C. Allee, to whom the author is deeply indebted for friendly guidance. Dr. C. M. Breder, Karl P. Schmidt, and Clifford H. Pope read the manuscript and contributed much helpful criticism.

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are well known in birds and mammals, but few thorough studies have yet been made. It is difficult to define leadership so as to exclude social facilitation, which Allee (1947) considers to be "any increment in frequency, intensity or complexity of behavior of one individual resulting from the presence of another." A common example of this effect is the consumption of more food by animals in groups than when isolated. Leadership seems to be a special case of facilitation, in which one animal sets the pace of group activity or initiates changes in it of various kinds.

An urgent problem in this area of sociology is the accumulation of data concerning the social behavior of lower vertebrates. On the well-grounded assumption that mammalian and avian behavior patterns stem from those of reptilian ancestors and that homologies can be drawn with the inheritable behavior of amphibians and fishes, it would seem to be of fundamental importance to trace the social instincts by comparisons between the vertebrate classes. It is difficult and sometimes impossible to distinguish homologous from analogous behavior, but valid generalizations of broad significance can often be discerned (Allee, 1938, 1945; Noble, 1938, 1939*a*, *b*; Collias, 1944).

Early in the present study it was discovered that small groups of immature sunfish (*Lepomis cyanellus* Raf.) establish hierarchies but may also become organized territorially. A simple kind of leadership or facilitation of behavior was noted. Therefore, it has been possible to undertake an experimental analysis of the relations between these types of social organization.

THE ANIMALS AND THEIR TREATMENT

The experiments reported here were designed to investigate (1) the types of organization established by small groups of immature sunfish, (2) some of the factors that determine position in a hierarchy or other organization, and (3) the effects of certain formalized complications of the physical environment upon organization. In addition, control observations were made of the adult fighting and courtship patterns.

All the sunfish used in this study were collected from a small permanent pond in the Tinley Park Forest Preserve in Illinois. A wide range of sizes and probably of ages was found during the summer of 1944, and in the fall a stock was maintained in large aquaria. These fishes were used throughout the following year until early summer, when immature fishes again became abundant. Collections were made also in July and August, 1946.

Hubbs and Cooper (1935) state that green sunfish ordinarily do not reach sexual maturity until they are about 3 inches in length; they found very few of the second-summer fish to be mature. For my experiments, individuals were selected that ranged from about 0.75 to 1.5 inches in standard length. It is almost certain that none of these was more than 1 year old. Sex could not be distinguished externally but was determined later at autopsy. Individuals could be identified through the circumstance that they had encysted parasites (trematodes) imbedded in skin and muscle and, in fact, all through the body; the parasites formed distinctive patterns of black-pigmented dots. The behavior of these fishes differed in no discernible way from that of noninfested sunfish collected from Hyde Lake, Illinois. Groups were selected in which the members could be identified readily and did not vary greatly in size.

Some of the experimental aquaria were situated in the Whitman Labora-

tory greenhouse, but the greater part of the study was performed in a large laboratory room, where the aquaria were kept under fluorescent lighting. Screens were arranged so as to minimize outside disturbance. The water used was Lake Michigan tap water, either heated or run through a charcoal filter to remove the toxic chlorine; it was changed in the aquaria every 4–7 days. The fishes were fed daily with enchytraeid worms, tubifex worms, or a cooked mixture of corn and soy meal, supplemented with liver or egg yolk. They grew best on live food but also did well on the substitutes.

In the experiments involving manipulation of environmental relations, single or multiple galvanized-wire partitions were set into the aquaria, each with a 2-inch square opening at the bottom. Details of procedure in the various experiments can most conveniently be given with the results.

BEHAVIOR PATTERNS

AGGRESSIVE BEHAVIOR

Sunfish that have settled their dominance relations do not ordinarily fight in the sense of showing the full fighting pattern. Instead, the dominant individuals drive their subordinates, that is, they make quick dashes at them, and the latter swim away, usually without being nipped. Occasionally, two may threaten by spreading their gill-covers and moving head-on toward each other; the threatened individual turns broadside and, with body held rigid, vibrates in a weaving fashion. An encounter seldom goes any further, unless the two are strangers.

The full fighting pattern of the immature sunfish cannot be distinguished in any particular from that of the adults. In both there is the same remarkable set of special pigmentation. The bony opercular flap has a large black spot, margined with salmon-red on the flexible portion. When fighting, the fish spreads its gill-covers, and this gesture carries the red-margined patch of black, which roughly resembles an eye, out to a position where it appears to the observer that the head has suddenly become very much enlarged. Another concentration of black pigment is present on the flexible portion of the dorsal fin and less conspicuously also on the anal fin. These spots darken and lighten with the apparent excitation of the fish. An actively fighting individual almost always has these spots intensified, but, if it loses the fight, the spots disappear. An interesting series of changes in eye color occurs during fighting. The iris is normally lightly pigmented, with a variable amount of red around the pupil. During an encounter, the red becomes more prominent, and a dark, inverted triangle appears above the pupil. If the fish loses, black pigment spreads to the rest of the iris and the red is obscured. A "black eye" is here an unfailing sign of defeat.

During the breeding season, male green sunfish develop fringes of white on the dorsal, caudal, anal, and pelvic fins. The latter have thickened anterior rays, although this does not seem to be connected with mating behavior. All the various tints of the male are strengthened at this time. The female, on the other hand, becomes less conspicuous. Females, and also subordinate individuals of either sex, exhibit a dorsoventral striping or banding of light and dark. There is some overlap in regard to the sex-specificity of these color patterns. Immature females often show the white margining of the fins but to a lesser extent than the males, and subordinate males are somewhat banded.

The initial move in the fighting pattern consists of a head-on advance toward the opponent, with gill-covers

erected; the latter responds with a seemingly defensive measure, namely, a weaving motion that appears to help in either avoiding or mitigating the force of a blow. The fishes take turns in advancing and weaving, but soon begin to nip furiously at head or body. Often they will catch onto each other's jaws and hold until one breaks away. When the fight is lengthy, the movements tend to become formalized, and as the opponents tire, they may move side by side, making weaving motions, or alternate in threatening, weaving, and nipping.

Females show the same fighting pattern as the males except when ready to spawn, at which time they are very subordinate.

REPRODUCTIVE BEHAVIOR

During the winter and spring (1944–45) a number of sunfish grew to mature

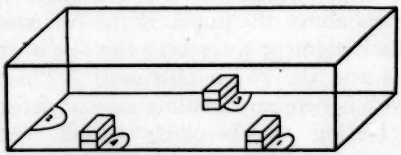

FIG. 1.—Breeding aquarium. Bricks were piled up to provide nest sites; the shorter sides of the aquarium are opaque.

size in the laboratory, and in May an attempt was made to induce them to breed. Two large aquaria (8×2×1.3 feet) were arranged for this purpose, as shown in Figure 1. The bottom was covered with about 1 inch of coarse sand, and bricks were placed at intervals, as indicated, to provide the kind of shielding barrier used by some species of sunfish (Breder, 1936).

In the first trial, two large males and what appeared to be a female were placed in one of the aquaria. After several days, a male dug an oval nest at position *1* and, sometime later, the other male nested at position *2*, at the opposite end of the aquarium. On May 23, Male 1 and the female spawned at position *1*, after which the female paid no further attention to the nest, while the male continued to guard the eggs. On June 2 the female was seen on the second nest, responding to courtship but not laying any eggs. On June 6, tiny fishes were detected; the adults were immediately removed in order to rear the young. Meanwhile, the same males were placed in the second aquarium, together with two other equally large males. To make conditions more nearly like the pond environment, 7 smaller fishes were added that were about half the size of the males. The first two males that had established nests at *1* and *2* in the first aquarium repeated their performance in the closely similar habitat in corresponding places. In addition, the two new large males built nests at positions *3* and *4* (Fig. 1). The group of smaller fishes did not nest but engaged in nipping and chasing among themselves; all were strictly subordinate to the large males.

Nest *3* was the least secure, and the male on it was subject to drives by Male 1 and Male 4. The latter especially exercised dominance over Male 3 in "nip-right" fashion so that for more than a month a kind of "territorial" hierarchy (Greenberg and Noble, 1944) could be detected, with Male 3 able to defend his nest only against the smaller fishes.

There was no sizable female available, and after a time it was observed that the borders of nest *1* were being progressively enlarged, almost to the extent of destruction of the nest. Finally, on July 9, a small female was observed to spawn with Male *2*; she was about half the size of the male, but the behavior of both was the same as in the mating seen earlier, and eggs were laid. Nesting behavior on the part of the males continued until the middle of August, when they began to move about more freely, with less attention to the nests.

The pattern of courtship and mating of *L. cyanellus* has not hitherto been recorded in the literature; it was very similar in all observed instances. While the male attempts to drive the female toward the nest and will make repeated excursions in her direction to do so, it is the female that determines when spawning will occur. The driving male behaves in the same manner as toward a male rival, but with much less intensity. The female finally circles the nest and comes to rest with head toward the angle made by the bricks and the glass. Here the male moves to a position directly over her. As he does so, the pair surge together into the corner, the male pressing on the back of the female; she turns ventral side up, rising upward; as her belly approaches that of the male, eggs are seen to be emitted from the genital opening. The pair move back and forth in the corner; at each movement the female presses her belly upward, close to the male's cloacal opening, and small numbers of eggs are expressed, to fall on the sides of the nest, on the plants bordering it, and in the nest itself. Sperm could not be seen, but fertilization undoubtedly occurred, since the eggs later hatched. The pair may stop and circle the nest, with the male at the outside, herding the female toward the corner. At intervals, he nips her and she leaves the nest, only to return after a short time. When the female is out of the nest, the male enters the corner head-first, and makes vibratory fanning movements with his tail.

During the various experiments, the immature fishes never showed the complete courtship pattern. In a single instance, observed from July 26 to August 12, 1945, a small male behaved in a peculiar fashion on a territory, and some phases of sexual behavior were detected such as circling a particular spot, driving others toward it, and moving above another fish. This was an exceptional observation.

TABLE 1

SUMMARY OF DATA CONCERNING 44 GROUPS OF 4 SUNFISH EACH, KEPT IN SMALL, BARE AQUARIA (10×8×8.5 INCHES) CONTAINING 4 LITERS OF WATER

	Expt. I	Expt. II	Expt. III	Expt. IV	Expt. V	Totals
Number of aquaria	8	8	8	8	12	44
Duration in calendar days	19	19	25	22	22	.....
Number of observation days	15	17	17	15	14	.....
Length of observation period (in minutes)	10	10	15	15	15	.....
Total recorded drives	4,258	3,733	7,707	5,592	10,244	31,534
Av. drives per group/hour	240*	164.6	226.6	186.4	244	214.6

* Group 5 with four territories was omitted because too few drives were recorded (62).

TYPES OF SOCIAL ORGANIZATION

The first series of experiments involved forty-four groups of four sunfish each, in small, bare aquaria (10 × 8 × 8.5 inches) containing 4 liters of water. These groups were observed for 19–25 days, between October 2, 1944, and January 14, 1946 (Table 1).

In the majority of the groups (27) the fishes developed a dominance order or hierarchy. A single individual became dominant and was able to move unhindered over the whole aquarium and freely drive all the others, whereas a

second fish could drive the remaining two, and a third drove only the fourth or "omega" fish. Such relations were often established by the very first day of observation and persisted, except for temporary reversals, during most of the observation period.

In the remaining seventeen aquaria, two or more individuals defended territories. In four cases, two fishes held territory, and the others were not only subordinate to them but were also organized in a hierarchical relationship. In twelve cases, three of the four held territories, while the fourth was subordinate to all. In the one unusual case, all four members of a group succeeded in holding territories; the lowest-ranking fish was the last to establish itself. Further details concerning these territories will be given below.

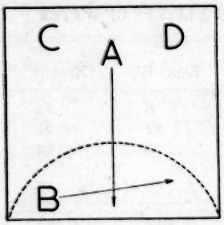

FIG. 2.—"Partial" territory. *A* is dominant in the whole aquarium, while *B* defends a small area against all but *A*.

It was impracticable to distinguish cases of a single fish holding territory from those in which a despot ruled the entire aquarium. With a floor space of about half a square foot, the dominant fish frequently darted across the entire area to attack a subordinate. Territoriality was clear enough, however, when two fishes threatened each other, neither dominant, and subdivided the available space, although with a somewhat indefinite boundary. Perhaps all hierarchies in small laboratory aquaria should be considered as single territories held by the despot, but the concept of territory as "any defended area" implies boundaries, defended against trespass. In the case of a single territory, therefore, it would be necessary to show that the boundaries lie within the aquarium walls, i.e., that the dominant fish voluntarily restricted its defensive reactions to only part of the total area. Presumably, in nature, boundaries are set by interaction between neighboring territory-holders, and available space is thus parceled out. Experimental conditions would seem to rule out the category of "single territory" from consideration in this series of experiments.

The twenty-seven hierarchies were complicated by "incipient" or "partial" territoriality. This was not difficult to recognize in seventeen instances; the fish ranking next to the despot was observed to take up residence in a corner or side of the aquarium and defended this area against trespass by all but the despot. The first-ranking fish could enter and drive this territory-holder, but the latter would often resist and would resume its defense when the despot left the immediate area. In the remaining ten cases this behavior was less evident and was not recorded as such.

The concept of "partial" territory differs somewhat from Diebschlag's (1941) terminology of "resting place" and "influence sphere." Figure 2 may serve to illustrate the kind of relationship observed in green sunfish. Here *A* is the despot, but the second-ranking fish, *B*, defends the area indicated by a dotted line whenever *A* does not interfere. Individuals *C* and *D* do not defend any specific place but rest more often in the indicated corners than elsewhere, and they can be said to have resting places. In this case there are no limits to the "influence sphere" or territory of *A*; the artificiality of aquarium conditions elim-

inates territory-holding neighbors and boundaries which would be delimited by mutual adjustment. Hence *A* does not hold the same kind of territory that exists when two or more fishes subdivide the aquarium space.

The partial territory of *B* may represent a step toward later equality with *A*, or it can result from a change in the status of *B*, which might previously have held full territory. In either circumstance, partial territory may be considered as intermediate between hierarchy and territory.

Even under our laboratory conditions, with oversimplification of habitat and limitations of space, territoriality is an outstanding phenomenon in the social life of immature sunfish. The territorial nesting behavior of adult male sunfish during the breeding season was known to naturalists long before its description by Reighard (1902), but there is no mention in the literature of territorial behavior in the immature fish of any species. The present experiment demonstrates that first-year green sunfish show aggressive behavior and defend territories, although not yet hormonally stimulated to exhibit reproductive behavior.

DESCRIPTION OF HIERARCHIES

Behavior on first encounter was variable, and usually the fishes spent some time becoming accustomed to the aquarium before reacting to one another. Soon one fish threatened another, eliciting either avoidance or counterthreat. Dominance was often decided by the early submission of a nonaggressive or smaller individual. If the fishes were fairly well matched, vigorous fights occurred at any time within the first hour and sometimes continued intermittently throughout the day. The winner showed the right to "drive" its subordinate, and the latter retreated. In most instances the subordinates became well trained to retreat and did so at the first move in their direction. As a result, the dominant fish seldom spread its gillcovers or showed other signs of the fighting pattern and rarely came close enough to nip. Such a "drive-order" contrasts with the "nip-order" described by Braddock (1945) for *Platypoecilus maculatus*.

Within a few days after the establishment of the hierarchy, its members settled down to incessant aggressive activity. In twenty-six groups,[3] the most dominant member drove its three subordinates at the average rate of 44.3 attacks per hour, the next-ranking individual drove its subordinates an average of 29.2 attacks per hour, the third-ranking fish drove its subordinate at a rate of 14.7 attacks per hour, and the lowest-ranking fish resisted its superiors at the average rate of 1.7 drives per hour (Table 3). In spite of this high frequency of aggressive interaction, the hierarchies were fairly stable. In seven of the above twenty-six groups, the rank order that pertained on the first day of observation remained unchanged, except for one or two temporary reversals. In another case the two upper ranks reversed after a few days and thereafter relations remained constant. A permanent upset of the two intermediate positions occurred in nine groups, whereas in four others the lowest fishes either exchanged places or never completely settled the dominance order. In four groups a single fish died and was replaced, with consequent changes in the hierarchy. In the remaining case, early instability of the three lower ranks was followed after 4 days by settling of relations.

The total drives made and received by fishes occupying each of the four ranks

[3] One group (No. 6, Expt. V), has been omitted from the total figures because the rank order was reversed several times (Table 7).

are compared in Table 2. The data for each hierarchy represent the longest stable interval during the observation period, and the nature of any major changes that took place is indicated. Table 3 presents the averages for each rank in terms of drives per hour of observation; the fishes occupying the alpha, or highest, positions drove their subordinates quite equally, on the average, regardless of rank. The beta, or second-ranking, fishes showed a tendency to drive their lower-ranking subordinates more often, although this was not statistically significant. The number of drives given are directly correlated with rank, whereas the drives received are inversely correlated.

A total of 582 reverse drives (subordinate driving superior) is recorded in Table 2. The fishes in each rank category were resisted in direct proportion to the rank of the subordinate. The orderly progression of numbers shown in Tables 2 and 3 suggests that the members of a hierarchy occupy steplike levels in dominance. The most dominant fish not only drives all the others but drives more frequently; resistance to the alpha fish is also weakest, as shown by the low frequency of return drives that it receives. The second-ranking fish drives its subordinates more actively than does the third-ranking fish and is resisted more actively by the latter than by omegas or lowest-ranking fish. The third-ranking individuals, while decisively dominating the omegas, also receive a large number of drives in return.

The majority of the hierarchy relations were of the stable "peck-right" type. Table 4 presents the frequency distribution of reverse drives in each of the six contact-pair relations. In sixty-four instances, the subordinate did not return the drives of its superior, while in thirty-five other cases it did so only once or twice. Relations between the despot fish and its three subordinates were more decisive than among the others; in the lower ranks, reciprocal driving was seen much more frequently (Table 2). In such cases one can consider the relation to be similar to the flexible "peck-dominance" of pigeons (Masure and Allee, 1934*a*). At any given time, either the dominant or the subordinate member of the contact-pair might be driving, with a better chance that it will be the dominant.

The stability of the relationships can also be shown by analyzing their duration in terms of percentage of total observation time. Table 5 gives the longest duration in calendar days of each of the six contact-pairs, arranging the members of the group by their rank assigned in Table 2; next to the duration is the percentage of the observation period that it represents. It may be seen that dominance was quite stable; the alpha rank was most consistently maintained, remaining unchanged for an average of 97 per cent of the observation period. In preparing this table a change was considered to have occurred whenever a subordinate drove its superior two or more times in excess of the number of times it was driven during the observation interval (10–15 minutes). Such changes were seldom permanent except during the early stages of hierarchy formation and for a few days afterward. The changes usually again were reversed in favor of the consistently dominant fish.

Table 6 presents an analysis of the duration of the entire hierarchy and of various subrelationships within it. The single pair-contacts lasted, on the average, 88 per cent of the observation period. Alpha dominated its subordinates very consistently (97 per cent of the time, on the average), whereas beta and gamma were not quite so effective (70 and 74 per cent, respectively). The hierarchy itself lasted

TABLE 2

TOTAL DRIVES GIVEN AND RECEIVED IN EACH OF THE 6 PAIR-CONTACTS COMPRISING A HIERARCHY OF 4 MEMBERS

GROUP NUMBER	PAIR-RELATIONS						DAYS DURATION	TYPE OF CHANGE*
	A—B	A—C	A—D	B—C	B—D	C—D		
	Experiment I							
1	79— 1	69— 0	49—0	35— 1	17— 2	6— 2	9	c
3	46— 1	128— 0	233—0	61— 2	150— 4	60— 1	19	a
4	111— 1	96— 0	97—0	46— 1	92— 6	12— 3	15	c
6	87— 2	185— 0	130—0	41— 5	87— 1	50— 10	19	a
7	82— 0	187— 0	163—0	49— 11	88— 9	34— 12	12	c
8	36— 3	79— 0	75—0	139— 0	191— 0	9— 0	19	a
	Experiment II							
17	41— 2	32— 0	30—0	59— 37	52— 12	30— 4	10	c
20	71— 5	81— 1	99—0	53— 11	42— 16	21— 14	15	c
21	64— 6	44— 3	40—4	52— 2	47— 2	7— 1	9	b
22	45—10	26— 0	62—1	38— 11	37— 4	11— 2	10	c
23	71— 0	111— 0	59—0	10— 7	23— 3	25— 8	14	e
24	32— 5	18— 1	30—0	43— 1	111— 1	6— 4	19	d
	Experiment III							
6	187— 0	96— 0	11—0	43— 10	23— 6	56— 9	16	c
12	364— 2	231— 0	199—0	137— 18	223— 1	169— 11	25	a
15	121— 6	206— 3	226—0	77— 4	83— 8	43— 15	25	a
18	237— 6	99— 0	198—3	105— 6	109— 34	33— 16	25	a
	Experiment IV							
3	172— 0	141— 0	96—0	27— 5	29— 7	23— 22	19	d
15	63— 0	54— 1	62—0	34— 3	43— 0	80— 0	8	c
	Experiment V							
5	139—10	93— 1	64—0	129— 7	117— 0	26— 0	16	f
7	173— 0	167— 0	106—0	19— 11	41— 1	33— 18	17	d, f
8	178— 0	157— 0	118—0	54— 9	35— 2	9— 2	22	a
18	97— 0	89— 0	59—0	37— 0	14— 9	35— 3	21	d
19	90— 1	145— 0	135—1	41— 12	31— 6	37— 15	15	f
20	154— 0	89— 0	176—0	23— 5	37— 4	14— 8	15	c
25	117— 0	150— 1	135—0	118— 2	122— 0	104— 2	15	d
26	126— 0	90— 0	87—0	91— 0	120— 0	17— 0	11	f
Totals	2,983—61	2,863—11	2,739—9	1,561—181	1,964—138	950—182	.....	.....

*a, No permanent changes in hierarchy; b, A—B pair-contact reversed permanently; c, B—C pair-contact reversed permanently; d, C—D pair-contact reversed or unsettled; e, early instability of lower three ranks, stable after 4 days; f, one fish died and was replaced, leading to changes in the hierarchy.

unchanged for an average of 54 per cent of the observation period.

In general, the hierarchies appeared to become more stable with time. There were some exceptions, in which permanent reversal of ranks occurred well along in the observations. In one case (Group 6, Expt. V), the fish ranking third at the start worked its way up to first and, in the process, brought about a series of changes in the hierarchy, as shown in Table 7. As L rose in rank, M dropped to omega. There was a parallel rise in rank of N to second place, and, as a result, O dropped to a fairly consistent third. All these fishes were females, and they were very closely matched in size at the start. At the end of the experiment, however, it was found that L and N had grown considerably more than M and O, so that a decided size discrepancy resulted.

This introduces the whole matter of position in the social hierarchy and growth, a subject that is reserved for a joint communication later with others

TABLE 3

AVERAGE RATES OF DRIVING PER HOUR OF OBSERVATION IN 26 HIERARCHIES OF FOUR MEMBERS

Rank of Driving Fish	Rank of Fish Driven			
	A	B	C	D
A	.....	44.6±2.4	44.1±1.4	44.1±3.9
B	0.97±0.23	.....	26.8±2.9	31.5±3.7
C	0.19±0.08	3.3±1.0	.....	14.7±2.1
D	0.15±0.09	2.2±0.5	2.7±1.1	.....

TABLE 4

FREQUENCY DISTRIBUTION OF NUMBER OF REVERSE DRIVES IN EACH OF THE 6 CONTACT-PAIRS OF 26 HIERARCHIES
(Pair-Relations Are in Order of Rank)

Number of Back-Drives	Frequency of Occurrence						Total
	A—B	A—C	A—D	B—C	B—D	C—D	
0	11	19	22	3	5	4	64
1	4	5	2	3	4	2	20
2	3	0	1	3	4	4	15
3	1	2	1	1	1	2	8
4	0	0	0	1	3	2	6
5	2	0	0	3	0	0	5
6	3	0	0	1	3	0	7
7	0	0	0	2	1	0	3
8	0	0	0	0	1	2	3
9	0	0	0	1	2	1	4
10	2	0	0	1	0	1	4
11	0	0	0	4	0	1	5
12	0	0	0	1	0	1	2
Over 12	0	0	0	2	2	6	10
Total pair-contacts	26	26	26	26	26	26	156

TABLE 5

LONGEST DURATION OF THE PAIR-CONTACTS IN 26 HIERARCHIES
(Expressed in Calendar Days and Percentage of Total Observation Period)

Group No.	A—B		A—C		A—D		B—C		B—D		C—D	
	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent
	Experiment I											
1	19	100	19	100	19	100	7	37	19	100	19	100
3	19	100	19	100	19	100	19	100	19	100	19	100
4	19	100	19	100	19	100	15	79	12	63	9	47
6	19	100	19	100	19	100	19	100	19	100	10	53
7	19	100	19	100	19	100	9	47	11	58	11	58
8	13	68	19	100	19	100	19	100	19	100	19	100
	Experiment II											
17	19	100	17	89	19	100	9	47	10	53	19	100
20	19	100	19	100	19	100	15	79	9	47	9	47
21	17	89	19	100	19	100	18	95	15	79	11	58
22	9	47	19	100	19	100	9	47	10	53	9	47
23	19	100	19	100	19	100	15	79	12	63	15	79
24	9	47	19	100	19	100	19	100	19	100	14	74
	Experiment III											
6	25	100	25	100	25	100	9	36	15	60	25	100
12	25	100	25	100	25	100	11	44	25	100	25	100
15	25	100	25	100	25	100	25	100	25	100	18	72
18	21	84	25	100	25	100	25	100	10	40	9	36
	Experiment IV											
3	22	100	22	100	22	100	22	100	22	100	10	45
15	22	100	22	100	22	100	8	36	22	100	22	100
	Experiment V											
5	14*	100	14*	100	14*	100	14	64	22	100	22	100
7	22	100	17*	100	22	100	11*	65	22	100	5*	30
8	22	100	22	100	22	100	22	100	22	100	22	100
18	22	100	22	100	22	100	22	100	16	73	19	87
19	22	100	22	100	16*	100	22	100	13*	59	13*	59
20	22	100	16	73	22	100	22	100	22	100	15	68
25	22	100	22	100	22	100	22	100	22	100	16	73
26	22	100	22	100	13*	100	22	100	13*	100	13*	100
Average duration in percentage	94		98.5		100		79		83		74	

* One member of group died.

who have been and are associated in this particular phase of the inquiry. Although the evidence is by no means sufficiently collected to allow a definitive statement, it is known that size is an important factor in determining social dominance in the green sunfish.

In connection with joint experiments on growth, a test was made to secure further evidence on the stability of these fish hierarchies. Eight groups were maintained without observation of their behavior for 33 days (March 23–May 4, 1945). After this period, the fishes in each aquarium were watched for nine daily observation periods, each lasting 10 minutes. Tables 8 and 9 give analyses of the drives recorded and the duration of the contact-pair relationships. Relatively few reverse drives were seen and most of the contact-pairs remained unchanged throughout the 10-day period.

The general picture of activity within the sunfish hierarchy is fairly clear. After a time, relations tend to become formalized. The alpha fish, if its subordinates are all unaggressive, will drive at random any fish it comes across, and the omega member of the group simply avoids the approach of any of the others.

TABLE 6

DURATION OF THE 26 HIERARCHIES AND OF VARIOUS INTERNAL SUBRELATIONSHIPS

Duration of Observation Period (Per Cent)	One Fish Dominates One Other	Alpha Dominates Three Others	Beta Dominates the Two Others	Gamma Dominates Delta	Four Fishes Maintain Same Hierarchy
1– 10	0	0	0	0	0
11– 20	0	0	0	0	0
21– 30	1	0	1	1	3
31– 40	4	0	3	1	4
41– 50	11	2	4	3	5
51– 60	10	0	5	5	6
61– 70	5	1	2	1	3
71– 80	10	1	1	4	2
81– 90	5	2	0	1	0
91–100	110	20	10	10	3
Total cases	156	26	26	26	26
Average duration in percentage	88.1	97	70	74	54

TABLE 7

DAILY RANK ORDER IN A HIERARCHY WHICH SHOWED SEVERAL REVERSALS

Rank	1/14	1/16	1/19	1/20	1/21	1/24	1/25	1/27	1/28	1/29	1/31	2/2	2/3	2/4
1	M	M	M	M	L	L	L	L	L	L	L	L	L	L
2	O	O	O	L	O	N	N	*	†	N	N	N	N	O
3	L—N	L	L	O	N	O	O	*	†	O	M	O	O	N
4	L—N	N	N	N	M	M	M	*	†	M	O	M	M	M

* Triangle situation:

† Only one contact seen (N drove O once).

If the second-ranking fish happens to be active and aggressive, it may be as much or more of a tyrant than its superior. It then begins to approach equality with the latter, which may drive it infrequently.

Conditioning up and down the scale has been observed within the hierarchy. Frequently, a second- or third-ranking fish, after repeatedly driving a subordinate, may then challenge a superior, usually with disastrous results. Conversely, repeated attacks by alpha may weaken the resistance of beta or gamma to drives by the others, leading to temporary changes in position.

An interesting example of conditioning is the phenomenon here called the "substitution" effect. In several cases the lowest-ranking fish was observed to take

TABLE 8

TOTAL DRIVES RECORDED IN EACH OF THE PAIR-RELATIONS OF 8 HIERARCHIES MAINTAINED FOR 43 CALENDAR DAYS AND OBSERVED ONLY DURING LAST 10 CALENDAR DAYS (EXPT. VI)

Group No.	Pair-Relations in Order of Rank					
	A—B	A—C	A—D	B—C	B—D	C—D
1	78—1	49—0	36—0	29—13	28—2	62—4
2	56—1	63—0	74—0	64—3	108—0	31—0
3	44—0	83—0	62—0	10—14	18—0	91—1
4	38—1	46—0	37—0	21—6	70—0	33—0
5	113—3	72—0	47—0	34—0	24—0	2—1
6*	14—0	89—0	68—0	54—0	45—0	13—4
7	75—0	70—0	77—0	49—0	46—3	9—4
8	10—2	45—10	32—0	4—3	6—0	8—0
Totals	428—8	517—10	433—0	265—39	345—5	249—14

*Two territories, held by A and B.

TABLE 9

LONGEST DURATION OF PAIR-RELATIONS IN HIERARCHIES OF TABLE 8

Group No.	A—B		A—C		A—D		B—C		B—D		C—D	
	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent	Days	Per Cent
1	10	100	10	100	10	100	3	30	10	100	10	100
2	10	100	10	100	10	100	10	100	10	100	10	100
3	10	100	10	100	10	100	4	40	10	100	10	100
4	10	100	10	100	10	100	9	90	10	100	10	100
5	10	100	10	100	10	100	10	100	10	100	10	100
6*	10	100	10	100	10	100	10	100	10	100	5	50
7	10	100	10	100	10	100	10	100	10	100	9	90
8	10	100	10	100	10	100	10	100	10	100	10	100
Average duration in percentage	100		100		100		82.5		100		92.5	

*Two territories, held by A and B.

advantage of the tendency of beta and gamma to flee the approach of the despot; it would dash at them, and they would run, unless they turned around to fight back, and then they would drive omega away with ease. Some of the reverse drives recorded in Table 2 undoubtedly can be traced to this kind of substitution, which also was performed by the others.

FACTORS IN DOMINANCE

Among the factors that can be postulated as affecting the outcome of a fight or the place of an individual in a rank order, sex, size, and previous experience or conditioning lend themselves most readily to experimentation. Although the sex of the immature fish could not be told externally, it was possible to distinguish the sex composition of the groups at autopsy by macroscopic inspection of the gonads (method checked by cross-section of gonads). In Experiments II–V, distribution of the sexes was determined and found to be 78 males to 66 females. Additional data on the effect of sex on hierarchy position were also secured from Experiment VI and from two other sets of ten groups each, observed under the same conditions as the others, but for a shorter time—August 6–August 10 and August 29–September 6. These two sets were controls for an experiment on the effect of screen partitions on the form of social organization (to be reported below). From these eight sets of experiments, totaling seventy-two groups, sixteen can be selected that fulfil the following conditions: first, they were hierarchies, not complicated by established territory; and, second, they consisted of two males and two females. In these sixteen critical cases, the alpha rank was held by fourteen males and two females, the beta rank by eleven males and five females, the gamma rank was held by four males and twelve females, and the omega or lowest rank by three males and thirteen females. This indicates a correlation between maleness and high social rank.

During the early stages of formation of a hierarchy, the relative size of the fish is an important factor. Large sunfish dominate much smaller individuals without resistance by the latter; even within the minor range of size differences that were present, the larger fishes regularly occupied the higher ranks. Since both sex and size are factors in dominance, the question arises as to how they interact. To what extent does larger size overcome the handicap of femaleness? Some indication can be secured by reexamining the sixteen cases already discussed as critical for the sex factor. There are ninety-six pair-relations represented, of which two-thirds are heterosexual and a third unisexual. In sixteen male-male contact-pairs, the dominant individual was the larger in eleven cases; the dominant female was the larger in nine of the sixteen female-female pair-contacts. In sixty-four heterosexual contacts, the dominant male was larger in forty-four of fifty-three cases, while the dominant female was larger in seven of eleven cases. The fact that so many of the males that dominated females were also larger somewhat confuses the picture. It is possible, although not probable, that this may be a systematic error arising from the composition of the field collections. Hubbs and Cooper (1935) found that male sunfish in nature grow faster than females but are not significantly larger until their third summer. Our laboratory experiments made in another connection demonstrate a significantly faster rate of growth of males over females in the first and second years.

Hubbs and Cooper suggest that "the

increased growth of the males has been of selectional significance, enabling them to ward off enemies from the nests which they guard so pugnaciously." This hypothesis may be restated by defining enemies to include intra-specific male rivals; and the selection would then be in part also intra-specific sexual selection of the Darwinian type (Huxley, 1938).

The effect of conditioning on the members of a hierarchy has already been mentioned. The members of a group develop stereotyped reactions to one another and to the aquarium; place conditioning is especially prominent and may lead to territory; but, even when no definite territories exist, each fish tends to stay more often in a particular place than in others. The subordinates have certain positions in which they are safer from attack than elsewhere and also develop movement habits that take them out of the path of their superiors in rank.

When a new fish is introduced to replace a member of the group, it is quickly relegated to the bottom rank, unless it is much larger. The newcomer transgresses upon all the relations that have developed and at first is driven by each resident fish. After a while, however, the newcomer begins to assert itself and may advance in rank. Thus, in Group 5, Experiment V, a fish introduced as replacement for the omega rose to the alpha position in a few days.

From May 9 to May 11, 1945, the dominant individuals in the hierarchy groups of Experiment VI were interchanged to see what rank a long-established alpha fish would take in a strange hierarchy. The alphas that were exchanged differed in size by little more than 1 mm. in three instances but were somewhat unequal in the fourth case. Since these individuals were long accustomed to be dominant, it was of interest to see whether they could substitute mechanically for each other or whether they would meet with resistance.

After the exchange of the dominant fish in Groups 1 and 2, both were observed together for 1½ hours. For the first 15 minutes, the second-ranking fish in Group 2 took over, and the newcomer went to the bottom of the hierarchy; soon, however, it began to return challenges and within another 20 minutes it had succeeded in defeating all the resident fish and became the despot. In the other aquarium the newly introduced fish at first made persistent efforts to get through the glass of the aquarium. During this time, it was repeatedly driven by the second-ranking fish and occasionally by the others. After a while it began to resist attack, first defeating the second-ranking fish and then dominating the group.

In the second exchange, between Groups 3 and 4, events proceeded in a much similar fashion. The alphas, when placed in strange aquaria, at first did not resist attack and were driven by the residents. Within 45 minutes, however, each had won to the top.

The alpha fishes of Groups 5 and 6 were unequal in size, measuring 47.3 and 39.7 mm., respectively. When they were interchanged, the smaller went to the bottom of the new hierarchy and stayed there for 2 hours and 15 minutes of observation. In the other group the large newcomer was not active, and, although he was driven a few times and later drove once in return, no conclusive results were secured.

In the fourth exchange the two alphas were of about the same size. After 11 minutes one of them was dominant in its new group; by 31 minutes, the other had asserted itself and had become dominant. Neither encountered much opposition.

Thus in three cases the dominant fishes of two groups were exchanged and

replaced each other, rising to top rank in 35, 51, 45, 45, 11, and 31 minutes. In the remaining case one of the alphas was too small for its new group, becoming omega, and the other was unaggressive in the new situation. All these fishes, when returned to their old places, almost immediately resumed their original positions. During their absence fights occurred among the subordinates, but there were no reversals in rank.

This apparent ability of a long-dominant fish to retain its dominance even in a strange group probably resulted as much from the conditioning of the subordinates as from its own previous experience. These tests are too few to be taken as approaching conclusive results, but they do indicate the importance of the factor of experience or conditioning.

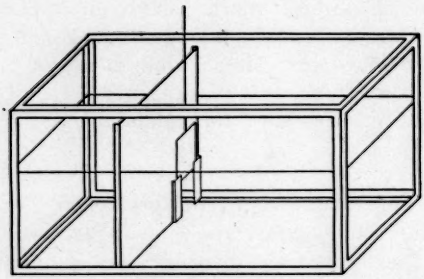

FIG. 3.—Maze used in tests of relations between leadership and hierarchy. Adapted from Welty (1934). Aquarium measures 16×10×10 inches.

HIERARCHY AND GROUP LEADERSHIP

Although the green sunfish does not school as compactly as certain other species, there are some indications of mass facilitation of activity. The fishes tend to follow one another for variable distances, and any quick movement of one fish attracts the others toward it. A fish performing a "scratch reflex" (scraping the sides of the body against any suitable object, an action that is frequently repeated) is often followed for a short distance. The first to see food darts forward and is immediately followed. A bit of inedible material resembling food, when mouthed and spit out by one fish, is then in turn mouthed by other individuals. A subordinate driven by one territory-holding fish is then very often set upon by the adjacent territory-holder, even from some distance. Similarly, an attack on a subordinate by one of its superiors tends to draw upon it attacks by the others.

Among those vertebrate groups in which hierarchy is important, the dominant individual may be the leader, or may not, as in chickens (Fischel, 1927). From a comparative point of view, it is of considerable interest to know whether any relation between dominance and leadership prevails in teleost fishes. A simple test was designed, based on the learning experiments performed by Welty (1934) with goldfish. Six aquaria (16×10×10 inches) were arranged on a water table, the broad side toward the observer, and a wire-screen partition with gate was set about a third of the way to one side (Fig. 3). The gate was closed by a sliding door, opened from behind a burlap screen by means of a pulley. As part of the conditioning stimulus complex, a 7-watt electric bulb, set into a small shielding box, was placed in front of the larger compartment of the aquarium. On June 16, four fishes were introduced into the large compartment of each aquarium and were observed at intervals until June 19, when all the groups had become organized as hierarchies.

A trial consisted of first turning on the light and then opening the door as gently as possible, when all the fish were about an equal distance from the gate. As soon as a fish came through into the small compartment, it was fed by dropping enchytraeid worms into the water. The

following data were recorded: (1) whether the alpha fish "led" through the maze, (2) the learning time of the first fish through the maze, and (3) the time of the slowest fish at each trial.

As shown in Table 10, the dominant fish led in 47 per cent of the trials; this is about twice the frequency expected by chance. It led more often in the first three trials (first in eleven of eighteen cases) than in the last seven trials (first in seventeen of forty-two cases). After the early trials it became a race and somewhat a matter of chance which fish reached the door first, and the dominant fish was handicapped because the observer opened the door when alpha was in the middle of the group, in neither a favored nor a back position. As a result, the subordinate nearest the partition had a shorter distance to travel and often darted through as soon as the door was opened.

The stimuli of turning on the light and opening the gate were separated by a brief interval, usually less than 30 seconds, while the observer waited for the fishes to be in fair position for the test. During this interval, increased activity and intensified driving were observed. The fishes soon became conditioned to approach the light, and they moved about much more than usual. The dominant fishes drove more actively and so did the higher-ranking subordinates.

As seen in Table 10, the learning time

TABLE 10

LEARNING TIMES (IN SECONDS) OF THE FIRST AND LAST FISH IN GROUPS OF 4 IN WELTY-TYPE MAZE

Trials	Groups					
	1	2	3	4	5	6
1	370* / 405	125* / 285	140 / 170	160 / 405	120 / 395	60* / 75
2	130 / 140	80* / 105	55* / 65	50 / 85	10* / 20	10 / 25
3	30 / 45	30* / 35	60* / 65	10* / 20	10 / 15	10* / 30
4	15 / 30	5 / 20	30 / 35	5 / 20	10* / 20	10* / 15
5	2 / 10	5* / 10	2 / 20	1 / 5	10* / 15	5 / 10
6	2* / 5	2* / 5	5* / 10	2* / 20	10* / 20	2* / 5
7	2* / 5	15 / 75	2* / 15	1 / 5	2 / 4	2 / 4
8	1* / 30	1 / 10	1 / 5	1 / 5	1 / 2	5 / 40
9	1 / 3	1 / 15	1 / 2	2 / 10	1 / 2	1* / 2
10	1 / 10	1* / 2	2* / 30	1* / 2	1* / 3	2 / 5

* The dominant fish in the group came through gate first.

dropped steeply at the second and third trials. These are not the records of individual fishes but of the fastest and slowest through the maze at each trial. An important element in bringing the slower members of the group through the maze was the feeding activity and visual stimulus of the food. In the early trials a slow fish sometimes stopped at the partition near the food, finding the door by moving along the screen. After the first three trials, however, the members of the group usually came through so closely together that they fed at the same time.

Welty (1934) found that grouped goldfish learned more rapidly than did isolated ones. His work on this point has not received the experimental repetition[4] that its importance justifies; so we tried isolated sunfish in the same maze situation as a check on the speed of learning. Twelve sunfish were placed in separate mazes and tested for learning in the same fashion as the groups were. An interval of 20 minutes was allowed for the fish to come through the gate and be fed. After that time they were lured through by dropping enchytraeid worms near the doorway. Table 11 presents the learning times in seven trials. Six individuals exhibited regular learning curves, two (Nos. 3 and 11) improved markedly after the fourth trial, and four (Nos. 1, 2, 4, and 12) gave irregular performances. Much of the "learning" shown by the sunfish seemed to be the overcoming of negative reactions to elements in the stimulus situation. The light, movements of the door, and vibrations in the water tended to startle the isolated fishes more than they did the grouped ones. At any rate, the performance of the grouped fishes was decidedly superior to that of the isolated ones (Fig. 4).

TABLE 11

LEARNING TIMES (IN SECONDS) OF 12 ISOLATED SUNFISH IN WELTY-TYPE MAZE

Isolates	Trials						
	1	2	3	4	5	6	7
1	1,200	298	350	666	426	232	1,187
2	1,200	1,175	365	1,200	430	344	1,200
3	590	120	729	700	67	7	11
4	379	75	235	385	54	169	212
5	653	617	249	22	54	26	20
6	1,167	178	144	6	22	3	2
7	1,200	761	38	9	48	5	12
8	1,200	100	58	13	5	12	10
9	426	1,095	123	23	24	40	12
10	745	86	122	14	25	7	10
11	170	196	204	168	7	18	18
12	1,200	185	570	83	512	286	12

The bearing of these facts on the problem of the relation between dominance and leadership is not wholly clear. There is group facilitation of learning, which may result from imitation, leadership-followership, or the greater "freedom from fear" afforded by group membership. Under our aquarium conditions the dominant fish tended to "lead" through the maze, at least in the early trials, which are most significant for learning. It is not clear whether this superiority

[4] Except for unpublished reports from students in Dr. Allee's classes in animal behavior.

reflects superior innate learning ability. In the early trials the dominant fish tended to explore the door and smaller compartment before the others. The dominant member of a group moves more freely in an aquarium, and this may explain in part the alert behavior of the dominant in a learning situation.

DESCRIPTION OF TERRITORIES

Seventeen of the forty-four groups comprising Experiments I–V (Table 1) became organized along territorial lines. In nine of these seventeen groups, some or all of the areas were claimed before the start of observation. A detailed record of the manner of organization of territories was secured in two cases (Groups 27 and 28, Experiment V), and a less complete account can be given of six others. The history of certain of these groups is presented in some detail because it provides an insight into the nature of territoriality.

The four members of Group 28 engaged in round-robin fights, B defeating D, and C dominating A. Soon B emerged as the despot by winning an encounter with C, the latter retaining the ability to drive A. Next, C was observed in a corner of the aquarium, which it defended against all except the despot, B. This behavior has already been defined as partial territory (p. 272). A half-hour passed, with no discernible change, and then B and D again fought; neither could dominate, but D tended to withdraw to one corner, while B remained at the opposite side (Fig. 5). Then there occurred a change in dominance between A and C; the former defeated C and took its place in the corner. After a while B challenged A, and they fought vigorously; but neither seemed to give way, and A retained its corner position. A and D challenged each other repeatedly and several times exchanged nips, neither achieving mastery. Soon it became clear that three territories existed in the aquarium and that C was omega or subordinate to all.

An interesting change was seen the following day. The three territories still remained, but D had become the most aggressive, with the largest territory at one side of the aquarium (Fig. 6), while A and B had shifted so that they occupied adjacent corners opposite D. These positions were kept for the rest of the 22 days of observation. The later history of this group is discussed in part in another section (p. 288). Fish A was the least aggressive of the territory-holders, and, as a result, C was repeatedly driven into A's territory and tended to find an insecure resting place there. Thus A drove the omega actually more often than did the other fishes; nevertheless, by January 24, C was resting mainly in A's territory when exhausted, and D often in-

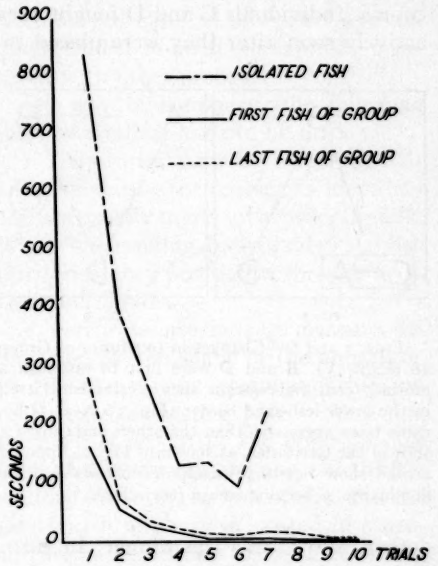

FIG. 4.—Average learning curves of isolated and grouped fishes in maze shown in Fig. 3.

vaded that area to attack omega. A few days later, it was seen that D's habit of invading had worked to the detriment of A, since D now also drove A; A and B were still in territorial relation to each other at the time. On February 2, A was also driven by B whenever forced into B's area. The omega died on February 4 and thereafter A was, in effect, the lowest-ranking member of the group.

At first, Group 27 followed a similar course. Individuals C and D fought very actively soon after they were placed to-

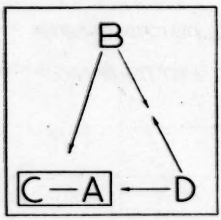

FIG. 5

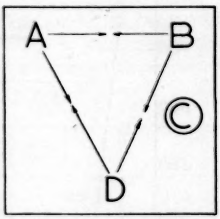

FIG. 6

FIGS. 5 and 6.—Changes in territories of Group 28 (Expt. V). *B* and *D* were first to establish a mutual, territorial relation; then *A* established itself in the lower left-hand corner (Fig. 5). Next *D* became more aggressive than the others and caused a shift of the territories, as shown in Fig. 6. Apposed arrows show territory; unapposed arrows indicate dominance. *C* became omega (encircled).

gether, and C was the winner. In turn, C defeated A and B and became the despot, in control of a large part of the aquarium. Next, B withdrew to a corner and defended it against the others. This corner was directly opposite the usual residence of C, and it appeared that the location of this territory was determined by the prior position of the despot. The following day, D had displaced B and thereafter held the corner territory.

Subsequently, the two territory-holders became very aggressive and kept A and B in one corner; they remained there until January 26, when an abrupt change took place: A drove B out of the corner, chasing it persistently (forty-nine times in 15 minutes); A was relatively unmolested (5 drives). Thereafter, A gradually won a small territory in the corner; on the other hand, B was driven relentlessly, and on February 6 it was dead.

The sequence of events is similar in these two cases. First, vigorous fights resulted in an unstable dominance order. The resting place occupied by the most dominant individual seemed to determine an opposite pole, at which several fish alternated, and ultimately one established territory there. When the third territory was seen, it was at an intermediate position.

Seven other groups were observed initially when they still were hierarchies; in five of these, territory was eventually taken up strictly in accordance with rank in the hierarchy. The beta individual became equal in rank with the alpha, and the two restricted their movements to separate areas. When a third territory also was held (in five of the total of eight groups), the gamma fish occupied it. In one exception the two highest members of the hierarchy first held territories; 9 days later, there occurred a reversal in rank of the two subordinates, and very soon thereafter the winner also took up a territory. Another exception arose when the lowest-ranking member of a hierarchy died and the new fish that replaced it succeeded in maintaining an area against the residents. The original alpha and the newcomer both drove the other two fishes, but not each other, and stayed in separate parts of the aquarium.

This behavior of a replacing fish was seen in two other instances. In Group 9 (Experiment III), B and D were first observed to hold territories, and C was omega. The next day, C was dead and was replaced with a fish that was 4 mm. larger than A and ½ mm. larger than B and D. That same day the newcomer was driven by both territory-holders but

not by A; the following day, the newcomer had become equal in rank with D, and 3 days later it held a territory alongside B and D. On the days immediately before the new fish acquired its territory, it drove omega twenty-eight and twenty-three times in two 15 minute observation intervals, chasing it into the paths of B and D, which, in turn, drove it repeatedly. It was my impression that in this manner the nonresident fish was building up an immunity from attack.

A comparable set of observations was made with Group 3 (Experiment III). At the very first observation, three territories were seen, with D as omega. Two weeks later, B lost its position and at the same time became subordinate to D. On that day, B was driven a total of one hundred and three times in 15 minutes of observation; it was dead the next day. The fish replacing B was the same size as the residents. It was attacked persistently by all, and evidently could not find a resting place in the aquarium. It was not reactive to the social relations that already existed, for example, the invisible boundaries and neutral areas, whereas D, also subject to attack, could retire to a certain corner and be safe for a while. The new fish was driven almost constantly by A and C, while D remained unmolested and even took part. On the following day, however, the newcomer had ousted D from its resting place in the former territory of B and was in process of taking over this area. Again, it was seen that in the short observation periods on consecutive days the newcomer drove omega twenty-six, twenty-nine, and seventeen times, seeming to force D out in front of it and thereby gaining a measure of immunity from attack. It held this territory until the end of observation, 5 days later.

Group 5 (Experiment I) was found to be organized into three territories at the start of observation; A, B, and D each stayed in a corner of the aquarium and sallied out to drive C or to challenge each other. Fish C found a resting place only in the remaining unoccupied corner. Three days later, C had succeeded in repulsing drives by first A and then B, on each side of it. From then on, the four fish were all equal. They challenged but seldom drove each other, except when one trespassed on another's territory. Each stayed close to its corner and activity tended to be at a minimum, compared with other groups.

By way of summary, the following three generalizations can be made:

1. Territories may be established at any time during the course of a hierarchy and are usually taken up in order of rank. When low-ranking individuals establish territories, they first defeat their immediate subordinates.

2. Territories are, in large measure, determined by the shape of the aquarium, i.e., the corners of a square aquarium are much used as resting places. A second territory is set up at the opposite pole from an already existing one, the third is equidistant from the two others, and the rare fourth territory is located in a neutral area with relation to the three others.

3. Territories are stable but may undergo changes such as elimination of one, transfer of ownership, shifts in position and boundaries, and variations in relative aggressiveness of the possessors.

In the foregoing accounts there is a suggestion of an important social function of the omega fish. Especially in the case of territories, the omega seems to be a sort of buffer, releasing tension between the high-ranking individuals. When a territory was about to be taken, attacks upon the omega may have been an effective part of the readjustment of the group.

This suggested role of the omega was

investigated in the following series of tests. At the end of Experiment IV (December 25), Groups 18 and 21 were retained because there were three firmly held territories in each. On January 1 the omegas were removed permanently, to see whether this would affect the territories; the groups were observed for 15 minutes immediately afterward. The territories were not disrupted, but there was a rise in the frequency of challenges. On the last day of previous observation (December 25) sixteen challenges were recorded in Group 18; after the removal of omega, there were twenty-seven. In Group 21 there were three challenges on December 25 and twenty-six after the removal of omega. The omegas were kept out of the aquaria overnight, and on the following day the two groups were again observed for 15 minutes each. In Group 18 there were twenty-five challenges, four full fights, and two drives, and in Group 21 there were thirty-eight challenges and five drives.

A new fish was introduced into each group, and it was observed for 15 minutes, after a 2-minute interval for adjustment to disturbance. There were eight challenges and four drives among the three resident members of Group 18, whereas they combined to drive the nonresident fish one hundred and fifty-seven times. In Group 21 the newcomer was driven two hundred and twenty-five times in 15 minutes, keeping the residents so busy that they challenged each other only six times and drove each other seven times, occasionally perhaps by error.

These tests show that either the omega or the nonresident fish draws upon itself the attacks of the territory-holding animals and thereby reduces tension between them. In the absence of other objects of attack, fishes that have territories close together, as in our aquaria, challenge each other, and tension may increase.

A further test was made with Groups 27 and 28 of Experiment VI. On February 4, the last day of that experiment, the omega of Group 28 died, and A, which earlier had held a territory but was now subordinate to D, came in for more than its usual share of attacks. Two days later, Group 28 was observed for 15 minutes, and during this time A was driven thirty-six times by B and eighty-two times by D. There were also eleven challenges between B and D. A new fish that had been isolated was added. The newcomer was in the same size range as the other three fishes. The group was observed for 30 minutes; during the first 15 minutes, D drove A only nine times, and B drove A just once, while B and D challenged each other six times. This was a considerable advance for A and a reduction in reactions between B and D. The latter drove the newcomer thirty-nine times, and B drove it thirteen times. The nonresident fish was forced to stay in the same corner with A, where it was driven least by B and D. In the next 15 minutes, A drove the newcomer twenty-one times and also vigorously resisted D, challenging it six times and once engaging in an active, though losing, encounter with both D and B. D drove A thirteen times, and B drove it once. There was no tension in the form of challenging between B and D. The new omega was driven ninety-six times by D and twenty-three times by B.

The omega was left in the aquarium for 24 hours, and the group was observed again for 15 minutes. The situation had improved even more for A, and it had won back its territory. It was driven only once by D and twice by B. The omega was driven thirty-three times by A, nineteen times by B, and fifty-eight times by

D. It appeared to the observer that A was driving the newcomer out in front and that it became a buffer between A and the aggressive D. Often, when D drove omega near to A, the latter, in turn, drove it out toward D's territory, and D's attention was taken up with omega, leaving A unmolested. The results, as described, indicate an important social function of the omega fish, in territorial organizations at any rate.

On February 6 the situation in Group 27 was similar to that described for Group 28; the omega had just died, and when the group was observed for 15 minutes, A was found to be the subordinate. Another fish (B′) of about the same size was added; it had been kept isolated (in an adjacent aquarium). The newcomer was immediately attacked by D, but it resisted vigorously; the two were equally matched, and after 8 minutes they separated, both exhausted. The new fish drifted near A, and the latter challenged and attacked it. B′ fought back and won, driving A out of its resting spot. Then B′ attacked C, and the resulting fight was indecisive. A short while later the newcomer attacked D, and another vicious fight ensued, with neither able to overcome the other. After 19 minutes, the newcomer had taken over A's territory and was on an equal footing with C and D. In the next 25 minutes, B′ alternately fought with C and then with D, always holding its own, and between fights it drove A as often as the others did. After a total of 35 minutes of observation, B′ was firmly established on A's territory, and because of B′'s belligerence D was displaced from its resting area, moving over to the adjacent corner. This situation still existed 24 hours later.

In this case the newcomer was very aggressive from the start and succeeded in withstanding the attacks of the two territory-holders. By ousting the weakened A, it gained a territory and was even able to extend it at the expense of D. Here the introduction of a new fish resulted in a marked deterioration of the position of A, but the same event in Group 28 brought about an improvement for a fish in a similar situation. The critical difference was the relative aggressiveness of the two newcomers, in proportion to that of the residents.

FACTORS INFLUENCING THE ESTABLISHMENT OF TERRITORY

When the space for territories is limited, adjacent individuals may divide this space unequally but still remain in balance at the boundaries. Aggressiveness diminishes with distance from the territorial center, and this phenomenon may explain, in part, the paradox of social equals in possession of disparate areas.

Territorial balance may be achieved at the first fighting encounter or shortly afterward. If established later, within a hierarchy, the territories are taken in order of rank and result from a rise in status of the highest-ranking subordinate to equality with the despot. In such cases, prior conditioning to an area may be involved in rise in dominance level. Another distinct possibility is that the tyrant may withdraw from a portion of its range, perhaps becoming in time less aggressive, and this may enable the second-ranking individual to establish a territory.

The factors which determine whether two animals will enter upon a territorial relation are those that decide their relative aggressiveness. The larger will usually dominate; this has been repeatedly observed. Sex may be critical, since males dominate females, but not necessarily decisive. All other matters, such as

health, strength, or previous experience —in fact, all the factors involved in dominance (Allee, 1945)—are also important in the establishment of territory. The aggressiveness of each animal is the result of the complex interactions of these factors, and, since territory is a balance, variations on each side are significant for the territorial relationship.

In fifteen of the seventeen cases of territory observed in Experiments I–V, sex was determined at autopsy; the ratio was thirty-three males to twenty-seven females. Twenty-one of the males and fifteen of the females held territory, and this was roughly in the same proportion as the relative frequency of the sexes in the total population. Seven of the fifteen females that held territory were the largest in their groups, whereas only two of the twenty-one males were the largest. This suggests that size was a more important factor for females than for males. That females of heterosexual groups held territory at all is evidence that sex alone is not decisive in this kind of dominance.

In five groups there was, at first, a hierarchy with a female as alpha. In each case the next-ranking fish was a smaller male that subsequently rose to equality with the female, and the two established territories. The rise in rank of the beta fish may have been correlated with maleness; but the female alpha fish, by virtue of its experience as alpha, may have retained sufficient advantage to prevent its complete overthrow. Conversely, in three cases, second-ranking females that were the largest in their groups rose to equality with the smaller alpha males but did not overthrow them and, instead, established territories. These cases point to the importance of previous experience in relation to territory.

There were two cases in which a smaller female initially held territory alongside two males but was ousted from her position and became omega. But in the majority of cases, territories held by males or females were stable.

Further evidence is derived from the twenty groups observed between August 5 and September 6, 1946, in connection with an experiment to be described below. Analysis of the data from these groups, which had a sex ratio of exactly 50:50, supports the points already made. Twenty-four males and eighteen females held territory; ten of the eighteen females and only seven of the twenty-four males were the largest in their groups. The relations between territoriality and sex or size held as a rule; the exceptions were due to undetermined factors.

Certain external influences modify territorialism; some of these are the configuration, subdivison, and amount of available space and the numbers of fishes present. A corollary is the factor of size; an area may be crowded for two large fishes but adequate for four much smaller ones. Preliminary experiments have been carried out with special attention to the factor of spatial subdivision.

With size of aquarium and number and size of fishes held constant, a wire-screen partition (with a 2-inch square gate at one corner) was introduced into the aquaria to divide them into adjoining compartments; in a series of twenty-seven experiments, the partition was placed about one-third of the way to one side. Twenty of the twenty-seven groups developed territories, and nineteen of them established two territories, in conformity with the arrangement of the aquarium. This is a considerably higher ratio than prevailed in Experiments I–V, with undivided aquaria. Since the groups in partitioned aquaria were maintained under somewhat different conditions of light and temperature (in a greenhouse, under natural illumination),

another set of experiments was performed, this time under the same conditions as in Experiments I–V. Two series were arranged, each consisting of ten aquaria with a partition set exactly in the middle, and alternating with these were ten control aquaria without the partitions. The first series was observed from August 5 to August 10, until stable relations had apparently been established; the second series was observed from August 29 to September 6. Table 12 presents the data with regard to types of social organization established. Fifteen of twenty groups in partitioned aquaria developed territories while only five of twenty control groups established territories.

The principal effect of the partitions was in relation to defense of boundaries. The gate became the site of interaction between the most aggressive of the fishes, alpha and beta occupying separate compartments, and the subdivision into territories appeared to be facilitated.

When the partition was set one-third of the way across the aquarium, the aggressiveness of the two territory-holders may have been correlated with amount of space. Frequently, the fish dominant in the large section kept all the others from entering it. In these cases the smaller compartment contained a typical hierarchy. Whenever the territory-holding fish in the smaller area drove a subordinate through the gate, it was promptly chased back again. After a time, movements tended to become stereotyped; the subordinate was driven with regularity and usually retired to a neutral position; but when it was evicted through the gate, the other territory-holder would be ready and waiting to drive it back. Each fish of the group played its role smoothly, and there seemed to be little friction; after a time the two territorial individuals seldom challenged each other and even came to rest close together on opposite sides of the gate.

Not all territorial relations remained so stable; occasionally the more aggressive individual became bolder and trespassed upon the territory of the other, at first entering a short distance to drive a subordinate; the two dominant animals threatened each other, and the intruder then retreated. In several cases the more aggressive fish also began to drive its neighbor and by stages reduced the latter's territory to a partial one. The despot kept one compartment clear for itself, but also entered the other compartment and attacked all the others. The subordinates could seldom be driven from the smaller space, because there they were relatively safer than in the larger space.

TABLE 12

EFFECT OF SUBDIVIDING THE AQUARIA WITH PARTITION AND GATE UPON TYPE OF SOCIAL ORGANIZATION IN GROUPS OF 4 SUNFISH

Series		Hierarchies	Territories	
			Two	Three
1	Experimental	3	6	1
	Control	7	2	1
2	Experimental	2	8	0
	Control	8	2	0

In the majority of cases the groups were first organized as hierarchies and then changed to territories after the second-ranking fish had become equal in status and dominant in one of the compartments. The second in rank resisted attack more and more vigorously, especially at the gate, upon entrance of the dominant fish, and soon the latter challenged only at the boundary. Usually the individual resident in the smaller space thus gained a territory, but occasionally

it was the other way around. That is, in some cases a fish kept the smaller space as its residence area but invaded the larger space to drive all the others. Then the second-ranking fish would progressively limit these invasions by challenges and fighting, until there existed two territories.

Essentially the same relationships prevailed in aquaria divided into two equal compartments. Here, too, it was evident that the more aggressive fish stayed alone in its compartment but often made

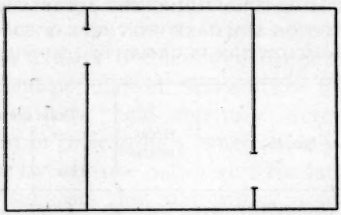

FIG. 7.—Wire-screen partitions divide aquarium into three compartments. Aquarium measures 16×10×10 inches.

minor forays into the adjacent area. There was an interesting difference in orientation of the territories; the control groups generally held territories dividing a front from a rear area, but the groups in experimental aquaria followed the left-right division provided by the partition.

At the end of this series of experiments each group was observed for 15 minutes, the partition was gently raised, and observations were taken for another 15 minutes. In a typical case, one of the two territory-holders (C) had kept its side clear of subordinates and, in consequence, had very little to do to maintain its area. It drove only four times (during the first observation period), as contrasted with the activity of its neighbor (B), which drove the two lower-ranking fishes forty-six times. After removal of the partition, C became very active and was able to drive the subordinates, since it now could reach them without trespassing at the door. C drove seventy-six times, and B drove sixty-six times; altogether, the total number of drives within the group rose from sixty-six to one hundred and fifty-seven in equal 15-minute periods of observation. At the same time, tension between B and C increased, as shown by eight challenges and eight drives between the two, as against none before. The territories were not abolished, but C proved itself to be more aggressive, continuing to keep its area free from subordinates, yet repeatedly invading B's area to drive them. The group returned to normal within a few minutes after the partition had been replaced.

Removal of the partitions in no case disrupted the territories, but there generally was an increase in challenging and driving between the territorial fishes. In four of the six groups there also followed an increased driving of subordinates by the two territory-holders. Other minor changes in status were observed; on replacing the partitions, the relations within the groups returned to normal.

The success of this method of influencing territoriality with partitions led to some preliminary trials with more complicated systems. In a set of larger aquaria (16 × 10 × 10 inches) two wire screens were introduced to provide three areas, the middle one being the largest (Fig. 7). Sixteen groups of four were observed in such aquaria; fourteen of them developed territories, an equal number being two- and three-territory situations. Hence it appears that provison of three compartments and more space resulted in establishment of a larger number of territories.

When there are three territories, the omega fish is at a decided disadvantage and may not survive, being shunted from

place to place and kept continually on the move. In one typical instance, it was seen to rest only when it retreated to a corner of the middle compartment, at the rear, and buried its head between the screen and the glass, facing away from its tormentor. Even there, it was sought out after a while and driven by all three territory-holders. The death or removal of the omega did not disrupt the territories, but subtle changes indicating an increase in tension could be noted. The fish threatened each other more often, and invasions increased in frequency. As in previous observations, the omega appeared to act as a buffer or releaser of tension between the territory-holding fishes.

There were interesting variations in the pattern of territorial division of space. Sometimes an aggressive individual took for itself two adjacent compartments and kept the others in the third. Here both a territory and hierarchy of three existed, as described for the two-compartment aquaria. In other instances the territorial fish remained in the side sections and the subordinates stayed in the middle one. The territory-holders would take turns entering the middle space to drive or would enter simultaneously while ignoring each other; in such cases individual recognition was obvious, for they often came to rest side by side, only to dart at another fish.

Another type of arrangement of partitions is shown in Figure 8. Only four groups were observed in these aquaria. In two cases, one fish held adjacent compartments, and two other fishes each held one area; the remaining fish was thus an omega. In another case one fish was so aggressive that it excluded all the others from three compartments, while a second fish maintained a territory in the fourth compartment. The fourth group was a hierarchy, with two fishes holding partial territories, subject to invasion by the dominant individual.

As shown in Figure 9, partitions may also be arranged so that four triangular niches open into a central square. Two groups of four fishes each, when placed in such an environment, established three and four territories. The fishes stayed in the outer niches, entering the central space either to threaten a neighbor or to drive the omega, in the case of the triple-territory situation.

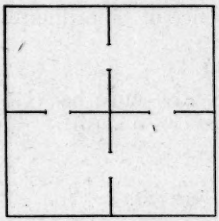

FIG. 8

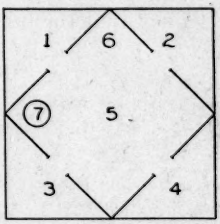

FIG. 9

FIG. 8.—Arrangement of wire-screen partitions to provide four communicating chambers. Square aquarium measures 10×10×12 inches.

FIG. 9.—Arrangement of wire-screen partitions with gates to provide multiple niches as a stimulus to territory. Square aquarium measures 10×10×12 inches.

Three trials were made with groups of seven fishes in such aquaria. The first trial yielded the most interesting example of territoriality so far recorded. Each corner was occupied by one individual, while a fifth was able to maintain itself in the middle section and resist the threats of the other four. A sixth fish held a partial territory within the angle made by two partitions (Fig. 9) but was subordinate to No. 5, as well as to the four that held corner territories.

The seventh fish (encircled) was omega and moved around the aquarium, driven from all directions. At the end of 3 days, the position of No. 5 had become untenable, and only the four residents in the side compartments still held territory. The three fishes in the center were

driven by these but still maintained a hierarchy. The group was almost constantly in motion; the territory-holders threatened each other, but it was obvious that they were inviolate in their own corners. First one and then another was the most aggressive in the central chamber and entered it farthest. Each fish appeared to be able to distinguish between a territorial and a nonterritorial fish, as well as perhaps individuals themselves, and drove the subordinates unerringly. Here, too, the impression was gained that the presence of subordinates lessened tension and diverted the attention of the territory-holders from each other. The group was under daily observation from August 27 through September 3, 1944, and less constantly through the rest of September; no essential change was observed.

TABLE 13

EFFECT OF SPACE ON TYPE OF SOCIAL ORGANIZATION IN GROUPS OF 4 SUNFISH

Size of Aquaria (In Inches)	Territories	Hierarchies	Total Drives
4·5× 4·5× 4·5.....	2	8	2,864
16 ×10 ×10.....	6	4	3,560
24 ×24 ×16.....	3	5	657

Two other groups of seven under the same conditions established only three territories per group. In both cases an aggressive individual held two adjacent corners, while two other fishes each held one corner; none of the remaining fishes held territory.

Although these experiments with more complex arrangements of niches are preliminary, they indicate that the condition of the physical environment with regard to subdivision into niches helps determine the location and extent of territories.

The amount of space available to a group is probably a factor in determining whether territories will be established, as shown by Shoemaker's (1939) study with canaries. A preliminary test of this factor in the sunfish was carried out from September 9 through September 29, 1945. From a stock that had been kept in the greenhouse for several weeks in extra-large aquaria (8 × 2 × 1.4 feet), twenty-eight groups (of four each) were selected, composed of fishes that were all about the same size. Ten groups were placed in 1-liter jars, ten others in medium-sized aquaria, and eight groups in much larger aquaria. Six rounds of fifteen minutes each were made between September 12 and September 29. Table 13 gives the total number of territories, hierarchies, and drives recorded under each of the three space conditions. In the matter of drives, there is a suggestion of an optimum space for the greatest number of reactions. Similarly, with regard to territory, the jars were decidedly too small, and the intermediate-sized space seemingly was most effective. These results suggest that for a given size of fish, there may be an optimum aquarium space for territoriality and/or frequency of aggressive interactions.

The effect of numbers present per unit of space has been investigated by Hixson (1946). Miss Hixson found an increase of frequency of territories in correlation with size of population, in the same volume of water. In eight groups of two fishes each, no territories were seen; in each of five groups of three, a single territory could be detected; in each of four groups of four, two territories were recorded; in three groups of six fish, two became organized as four territories, the other two as three territories; one of two groups of eight fish developed six territories, while the other developed five territories. This surprising correlation needs further study.

DISCUSSION

The literature on sunfish courtship and nesting behavior has been summarized in excellent fashion by Breder (1936). He finds that the species that have been studied are confined to a similar type of habitat and display essentially the same reproductive methods. *Lepomis cyanellus* has not been studied in the field, but in our laboratory its mating behavior followed closely the family pattern. An oval nest was constructed by the males and guarded both before and after eggs were laid, while the females participated only in egg-laying. As shown in Figure 1, all the nests were behind some opaque barrier. This closely resembles the nesting of *L. auritus* in a flowing stream, as reported by Breder (1936). Since no current existed in the aquaria, one must ascribe the position of the green sunfish nests to some other factor, possibly orientation to direct window lighting. The males entered the nests so that their heads pointed toward the angle made by the aquarium glass and the barrier, and they made sweeping movements with their tails to clear out an oval nest.

Reighard's (1902) brief discussion of the sunfish fighting pattern mentions the spreading of the gill-covers and the role of color display as an aid in driving away intruders, as well as perhaps in attracting the female. Sex differences in pigmentation and their significance in courtship were analyzed by Noble (1934) with *L. gibbosus* and by Breder (1936), with *L. auritus*. Although agreeing on the details of behavior, the authors differed with regard to the functions of male display in Darwinian sexual selection. Noble maintained that a "true" sexual selection exists in sunfish, based on the conspicuous colors of the males, which attract the attention of the females. Breder and Coates (1935) object to this hypothesis on the grounds that the sex ratio in sunfish is nearly equal and that hence such selection would be of no practical effect. Laboratory studies by Noble and Curtis (1939) with jewel fish seem to confirm the existence of a selection mechanism in which the color display of the males attracts the females. Greenberg and Noble (1944), experimenting with the lizard *Anolis*, also support the Darwinian hypothesis in modified form. A. E. Emerson suggests that natural selection acts not upon the individual male but upon the mating pair as a unit; the brilliant male display functions to integrate the pair more effectively. However, the Darwinian concept is concerned rather with which one of several competing males enters into the mating relation.

Adult male centrarchids have long been known to hold territories during the breeding season. Abbott (1894) observed that sunfish nests are often in a row or cluster: "The occupants of the several nests do not molest each other, and never intrude beyond the limits of their own 'homes.'" Lydell (1902) recorded the fact that male black bass, preparing to nest, fight actively; in a clear artificial pond an aggressive male will prevent any other bass from building a nest within 25–30 feet. However, nests of the smallmouth bass in the "natural water" were frequently built against a stone or log so as to be shielded on one side and no farther apart than 4 feet. Lydell constructed rectangular nest frames, placed them in spaced rows, and thereby increased the number of effective breeders. This method has become standard practice in bass culture (Langlois, 1936); it utilizes one factor in determining size of territory—the physical complexity or subdivison of an area.

Much of the literature on territorial behavior of fishes has been reviewed by Noble (1938). Defense of nesting site is common among oviparous fishes. That

territorial organization may not necessarily be associated with nesting is indicated by the observations of Meyer-Holzapfel (1941). She studied eleven South American Characinidae (*Hemigrammus caudovittatus*) of unknown sex in a large aquarium. (9 × 24.4 × 10 inches). The specimens averaged 7 cm. in length, presumably being adults. All except four established tri-dimensional territories; the four subordinates were relegated to "neutral areas," not defended by the other fishes. Contrary to Wunder (1930) and Breder (1936), who have found territoriality only during the mating season and held only by males, Meyer-Holzapfel suggests that there might have been a female among her territorial fishes and that they do not build nests. She concludes that territory is much more fundamental than hitherto thought.

It may be objected that, inasmuch as there were some fishes that did not have a territory, these may have been females. However, the present set of experiments with immature green sunfish of known sex supports her conclusions. Territoriality in the sunfish is independent of overt sex behavior or the genetic sex of immature individuals, although sex is a factor in deciding dominance or territorial relations in a contact-pair.

The factors influencing establishment of territory have already been discussed in part (p. 289). Underlying the behavior of adjacent territory-holders is a psychological balance at boundary lines, which manifests itself, in the sunfish, in the form of challenges or even nips but without any change in status. Although the fish must be equal at the boundaries, their territories may be decidedly unequal in extent, and this can be used as a measure of relative aggressiveness. Meyer-Holzapfel (1941) found that size of individuals was not important (within limits of ½–1 cm.), in the observed social organization of *Hemigrammus*. The sunfish seem to be more sensitive to size differences, but a minor disparity in size can be overruled by other elements of a complex combination of factors. These factors are the same whether the relation is territorial or hierarchical, except for additional influences, such as the effect of residence in an area, which may reinforce the dominance drive of one or both individuals.

The hierarchy had not been investigated in any centrarchid; in fact, for teleosts generally, published accounts of hierarchies are limited to partial summarizing statements by Noble (1939*a*, *b*), two abstracts by Noble and Borne (1938, 1940), and the investigation by Braddock (1945) of a viviparous poecilid. There is substantial disagreement between Noble and Braddock, although the species involved, *Xiphophorus helleri* and *Platypoecilus maculatus*, may be interbred in the laboratory. The most important discrepancy concerns the stability of hierarchies; Noble stated that rank orders could remain unchanged for several weeks, whereas Braddock's published results show the average duration of a hierarchy of four members to be little more than a day. Although both authors claimed that the organization of their species was of the same nature as the "peck-right" society of chickens, Noble's data have not been published in full and an examination of Braddock's (1945) tables leaves the decided impression that "peck-dominance" would better describe the shifting hierarchies of *Platypoecilus*.

The social hierarchy of the green sunfish is not so stable as that of chickens. Nevertheless, my findings agree with those of Noble and Borne; some hierarchies lasted without change for more than 3 weeks, and the majority of con-

tact pair relations were stable (Table 5). The frequency of "reverse drives," i.e., the subordinate, in turn, driving its superior, was generally low; in ninety-nine of one hundred and fifty-six contact-pairs, representing twenty-six full hierarchies, not more than two return drives were recorded during the entire period of observation (Table 4). Such one-sided dominance can be called the relatively absolute, or "peck-right," type, but there were frequently instances among the lower ranks in which subordinates returned a large number of drives. This condition of instability may arise from partial or incipient territory, the "confusion" effect (p. 279), unsettled dominance early in group formation, interference by the despot in the settling of dominance between the subordinates, and other irregularities. "Peck-dominance" in fishes may straighten out in time to "peck-right," as seen in Experiment VI; but, even after a long period, pair-contacts may still be indecisive.

In all but two of the one hundred and seventy-two groups that I observed, there was at least one subordinate individual; thus, where territory was held, hierarchal relations also existed, and the fishes defending areas drove the subordinates but not one another. Even in the groups organized solely as hierarchies, the drive to hold territory could be detected in the form of the partial territories held by one or more of the subordinates. Such semi-territories were seen in seventeen of the twenty-seven hierarchy groups of Experiments I–V.

These facts indicate that the principles of hierarchy and territory are not sharply separate but interplay in a variety of ways to shape the form of sunfish organization. It is highly probable that hierarchal relations of some sort exist in every instance of territory, but there is reason to believe that certain species that exhibit hierarchy are not at all territorial (Braddock, 1945). Whether territoriality is entirely absent even in these cases, or perhaps can be detected in modified form, needs to be further investigated.

A simple form of leadership can be discerned in groups of sunfish. It expresses itself in facilitation of various forms of behavior, especially feeding and the driving of subordinates. Leadership implies initiative and control of the pace of an activity, usually by one individual or alternately by several. A test for its presence in the sunfish was made by putting six groups through a learning situation to determine whether the most dominant individual in a group is also the leader. It was impracticable to secure the learning time for each individual; the six dominant fishes "led" in 47 per cent of the total trials (60), whereas the chance expectation is approximately one-half of this. Also a comparison of the average group performance with the learning shown by isolated sunfish (Fig. 3) indicates that there was a marked facilitation of learning in the groups.

Welty (1934) demonstrated that grouped goldfish are markedly superior to isolated ones in similar learning tests. It is possible to ascribe part of the group advantage to leadership, but it was observed also that isolated sunfish are more easily startled and become conditioned against elements of the test situation. Thus several isolated fishes gave erratic learning performances, while others did almost as well as the groups. The elimination of such variations may be part of the group benefit.

The tendency of the dominant fish to be the leader has far-reaching implications for further study. Settled hierarchies in chickens have greater survival value than groups kept constantly in reorganization (Guhl and Allee, 1944). If the dominant individual were also the

leader, it could contribute to group survival; this would balance some of the destructive individual advantages of aggressive drive.

The outstanding group value of aggressiveness in the sunfish probably centers about the importance of territory-holding in egg-laying, and the defense of eggs and young by the males. Fishes strung out along the shore on individual nests constitute questionable groups, but from an ecological point of view they are part of an integrated breeding aggregation. Territoriality spaces the nests and may make for more effective breeding.

The role of hierarchy in breeding survival is less obvious. A clue is afforded by the observations centering about the omega individual in a territorial situation. When three members of a group defend areas, the fourth almost always is severely punished, being driven at an excessive rate and often killed. Removal or death of the omega was followed by a rise in tension and increased frequency of aggressive contact among the territory-holders. Introduction of a newcomer centered attention upon it and reduced other aspects of intra-group friction. Thus it appears that these subordinates have a definite function in integrating the group.

SUMMARY

Groups of four immature sunfish (*L. cyanellus*), observed for periods of 19–25 days in small bare aquaria, maintain hierarchies or territories or both. Hierarchies are fairly stable and become progressively more so with time. Males usually dominate females, and the larger individuals tend to dominate smaller ones.

Among these fishes, hierarchy represents distinct levels of aggressiveness, whereas territoriality arises from a balance between almost equally aggressive individuals. This balance may be struck at first encounter; but, when it occurs later within a hierarchy, territories are first established by the two highest-ranking individuals, then by the third, and, rarely, by the lowest. Seventeen of forty-four groups kept in bare aquaria developed territoriality. In twelve cases, three of the four fishes held territories, while the fourth was subordinate to all. In one group each member held territory; here few aggressive contacts were observed.

Subordinates frequently defended areas against all but the most dominant fish. This "partial" territory seems intermediate between hierarchy and territory.

Groups tested in a simple maze-learning situation were superior to isolated controls. Leadership through the maze shifted at almost every trial, but the dominant individual tended also to be the "leader."

Aquaria divided into compartments by means of wire-screen partitions with square gates favored formation of territories, in direct proportion to the complexity of the subdivision.

Subordinates appear to lessen tension among territory-holding fishes; removal of the omega from aquaria with three territories markedly increased aggressive activity among those remaining, whereas the introduction of a new fish led to their attacking it instead of one another.

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OVARIAN ACTIVITY IN MAMMALS SUBSEQUENT TO CHEMICAL INJURY OF CORTEX[1]

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WITHIN the last twenty years one of us (C. R. M.) has several times attempted to destroy the cortex of mammalian ovaries in order to study the capacity for growth and development of the medullary components. These efforts were stimulated by such different aspects of ovarian morphology and physiology as (1) the demonstrated capacity of the ovary in the freemartin to transform into essentially a normal embryonic testicle, as revealed through the studies of Lillie (1916, 1917) and Willier (1921); (2) the development in human ovaries of such pathological structures as arrhenoblastomas, presumably derived from medullary components (Meyer, 1931); and (3) the completeness in submammalian vertebrates of transformation of ovarian medullary components into testicular tissue (*fish:* Essenburg, 1923, 1926; *Amphibia:* Witschi, 1939, 1942; Humphrey, 1942; *birds:* Benoit, 1923; Domm, 1929). Among mammals the freemartin stands almost alone in the development of characteristic seminiferous tubules from medullary cords in the ovary; the pig may also exhibit a somewhat similar tendency (Hughes, 1927, 1929). Cases of ovotestes, or naturally occurring hermaphrodites with ovary on one side and testis on the opposite, while known to occur in mammals, are insufficiently understood to be classified as testicular development from genetic ovaries. In the normal adult shrew most ovaries have been found to contain testis-like tubules that are believed to be homologous to seminiferous tubules (Wilcox and Mossman, 1945).

Theoretically, the problem of capacity of the ovarian medulla to develop into testicular tissue is approachable through experimental procedures that place the cortex at a disadvantage in development or that involve its complete removal. Presumably such measures should be more effective during development than after the gonad has attained maturity. Our previous unsuccessful attempts to remove the ovarian cortex have included the cutting-away of the ovarian surface with iridectomy scissors, application of contact cautery, diathermy treatments, and spot cautery from special electric circuits. These efforts were unsuccessful, since recovered tissues were characteristically ovarian or failed to show any frank developments in the direction of testis-like tubules. Still later experiments involved treatments of developing females with androgenic substances in attempts to duplicate freemartin conditions by application of known hormonal substances. In our hands, as well as in the case of all other investigators reporting the effects of androgens in developing female mammals, the expected freemartin conditions were not obtained; neither cortical destruction nor medullary stimulation has been realized in mammals by treatments with androgens.

Possibly the greatest development of

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medullary components into testis-like tubules in mammalian ovaries, as a result of experiment, was attained by the transplantation of embryonic ovaries to older male or female hosts by Buyse (1935) and by Moore and Price (1942). This development of medullary cords, however, fell far short of transformations realized by other investigators as a result of experiments with fish, Amphibia, or birds.

The present account of studies on mammalian ovaries is concerned with experiments performed on the ovaries of early postnatal rats, guinea pigs, cats, and opossums in a renewed attempt to remove the cortex at early periods in development in order to favor development of medullary components. This group of animals provides materials from very short-cycle females in rats, somewhat longer cycles in guinea pigs, still longer reproductive intervals in the cat, and a strictly seasonal-breeding animal in the opossum. It was anticipated that the cat and opossum might provide exceptionally favorable material for such a study because of the presence and considerable activity of medullary components described for the normal ovary, in which characteristic medullary follicles occur (Kingsbury, 1913; Morgan, 1943).

The procedure employed was the application of salicylic acid as a corrosive chemical substance to effect the destruction of the covering germinal epithelium along with varying amounts of underlying cortical tissue. The junior author perfected this method of procedure for the removal of the surface epidermis of feather papillae (Wang, 1943). On the feather papillae the epidermal layer is easily destroyed on intact, or surgically extirpated, papillae, and the relative roles of epidermal and dermal components in the formation of the feather were thus opened for study. In applying this technique to the ovarian problem it has been our aim to effect early cortical injury, or its complete removal, prior to the assumption of mature ovarian activity, and to study the modifications in the ovary that might develop subsequent to treatment.

Whereas the original purpose of conducting these experiments was a study of the reactions of the medullary components of the ovary, the second major ovarian problem of the relations of germinal epithelium activity to follicular development and the problem of the length of life of the egg and follicle have received our attention. The contrast is great between the older conception of the early segregation of a separate germinal line, with the formation of all ova accomplished during the embryonic period, and the more recent conception of a continuous formation of new germ cells and follicles throughout reproductive life. Such contrasts of ideas exist, therefore, as (1) the notion that, in the ovary of women, follicles may persist in an inactive state for a period of perhaps forty years and suddenly become active, differentiate, and ovulate; and (2) that the maximum life of a follicle and germ cell is very limited and compares favorably with that of a blood cell. Each point of view has been supported by many different investigators. According to Allen (1923), each oestrous period is associated with a new proliferation of germ cells and follicle cells from the germinal epithelium covering the ovary, as it is also associated with marked destruction of follicles present from a previous formation. Evans and Swezy (1931, p. 125) state for the rat: ". . . . it seems probable that the individual follicles have a functional life span of only a day or two, in all cases less than the length of the estrous cycle. . . . ." In the guinea pig they estimate

the growth from a primary follicle to large ones in 4 days and estimate the life-span of a follicle as 7–8 days; and for the cat (p. 182): ". . . . it seems evident that the life span of the greatest majority of ova may be measured by a few weeks at the longest and a few days at the shortest."

The problem of germ-cell origin and life-persistence of follicles is still a current one, and our observations on the treated ovaries of the four different species of mammals are believed to contribute some information germane to the problem. The rather large literature on these ovarian problems has been well reviewed in the articles of Allen (1923), Kingsbury (1913, 1938), Heys (1931), and others; and the interested reader is referred to them for more detailed discussion than will be undertaken here.

MATERIALS AND METHODS

The animals utilized in the study of experimentally treated ovaries were rats, guinea pigs, cats, and opossums born in our animal colony, except for a few kittens for which a definite date of birth could be assured. In all cases the ovaries were exposed through an incision in the body wall (lumbar region in rats, guinea pigs, and cats; mid-ventral for opossums) and were carefully elevated by a loop of thread around the base of the mesentery and walled off from other tissues by small, saline-saturated cotton packs or a dental rubber dam. The bursa was opened, where required, to expose the ovarian surface, and a solution of 25 per cent salicylic acid dissolved in Collodion Flexible (Merck) was spread thickly and evenly over the entire ovarian surface by means of a very small glass rod; care was taken to avoid contact of the solution with other tissues. Five to 10 minutes after application of the solution the film of collodion–salicylic acid was carefully removed under a Leitz binocular dissecting microscope by means of fine jeweler's forceps and saline-moistened cotton pellets. Some corroded surface tissue was usually adherent to the film on removal, and some exudation from exposed and injured capillaries was usually noted. The treatment was expected to permit surface penetration of the corrosive chemical substance and to destroy the germinal epithelium and varying depths of cortex, leaving the centrally located medulla essentially uninjured and vascularized by the intact blood vessels. From one to five treatments were given, depending upon the size of the ovary.

Without consideration of those animals dying prior to autopsy, 24 ovaries were recovered from 13 rats treated between the 8th and 20th postnatal days, with autopsy 54–354 days after treatment; 33 guinea-pig ovaries were recovered from 17 females treated between the 6th and 90th postnatal day, with autopsy from 93 to 343 days after treatment; 14 cat ovaries were recovered from 9 cats, treated between postnatal days 23 and 46, with autopsy from 98 to 320 days after treatment; and 21 opossum ovaries were recovered from 13 females treated between postnatal days 70 and 119, with autopsy from 36 to 341 days after treatment. Thus a total of 92 treated ovaries has been studied in serial section, representing varying periods of existence after treatment within the range of 36–354 days. The maximum age of animals at autopsy was 233 days for rats, 343 days for guinea pigs, 366 days for cats, and 428 days for opossums.

In some cases only one ovary received treatment, the second being removed as a normal control at the time of treatment; and in some cases in which both ovaries received treatment, one was subsequently removed at operation and the

female subjected to short treatments with an equine gonadotropin (gonadin) just prior to autopsy.

At autopsy the remaining portion of the ovary was usually firmly adherent to such tissues as fimbria, oviduct, epaxial muscle, etc.; and in a few cases no ovary could be found. In the rat, especially, fluid-filled cysts were frequently noted, some of which were hemorrhagic; these cysts in the ovary or in surrounding adherent tissues varied from ½ to 1 inch in diameter; and, whereas many were in the ovary, others proved to be cystic portions of the oviduct.

Table 1 presents some data on these treated ovaries, along with some notations of observations made from study of serial sections.

OBSERVATIONS ON TREATED OVARIES

In presenting our observations from the study of the 92 treated and serially sectioned ovaries the basic findings for each are briefly summarized in Table 1. For each of the four species studied, a sample of the histological observations will be presented in some detail as descriptions of one or more recovered ovaries, and generalized remarks will be included to cover the range of conditions encountered in each group.

The immediate effect of treatment was to leave the ovary in an obviously damaged condition. The entire ovarian surface, including the base of the mesovarium, appeared whitish or coagulated, and usually small serum exudates and occasional capillary hemorrhages on the surface were apparent. This was the case especially where second, third, or fourth applications had been made after the film of the previous treatment had been peeled off; these multiple treatments represented efforts to penetrate and destroy progressively deeper areas in the cortex. At the end of the treatment the whitish, surface-corroded organ always stood out prominently from surrounding untreated tissues.

RATS

To consider, first, the results noted in the serially sectioned rat ovaries, it should be emphasized that for the most part in rats, as well as in the three other species employed, cortical destruction was attempted at periods prior to the time of onset of ovulation and frequently prior to the appearance of a follicular antrum; in a few cases postpuberal ovaries were subjected to treatment. In the rat, on account of size, it was difficult to apply treatment to the ovaries earlier than a week to 10 days after birth. At this time numerous, mostly primordial, follicles are present, and antrum formation begins at approximately the 10th day after birth.

Rat No. 8 (ov. ser. 56A and B).—Both ovaries in this rat were subjected to treatment on day 9, with one application to each ovary of the collodion–salicylic acid solution. The remnants of the treated ovaries were removed at autopsy on day 362, or 353 days subsequent to treatment. Although oestrous cycles were not noted in detail, this animal showed a cornified vaginal smear just preceding autopsy. The uterus was normal in size and general appearance. Each ovary was firmly adherent to surrounding tissues, especially to oviducts, and each recovered mass contained ovarian tissue.

Each recovered ovary was totally devoid of germinal epithelium; small cysts, as well as some larger ones, were present. In the larger ovary, approximately 20 follicles were noted, consisting of 5 primordial follicles having a single-cell layer about the ovocyte, 7 follicles with 3–4-celled granulosa, and 8 follicles containing an antrum and an essentially normal-

TABLE 1

DATA ON RECOVERED OVARIES TREATED WITH COLLODION–SALICYLIC ACID

Animal Number	Ovary Series	Age in Days		Days Post-Treatment	Developing Follicles	Corpora Lutea	Testis-like Tubules
		At Treatment	At Autopsy				
				Rats			
4	6	20	74	54	Many	Recent; old	None
4	7	20	74	54	Many	Recent; old	None
3	5	20	94	74	Many	Recent; old	Few
3	11	20	99	79	Few	Pronounced	Few; poor
10	17	10	147	137	Many	Old	None
10	18	10	147	137	Many	Old	Few; poor
7	19	9	147	138	Many	Many	Few; poor
7	20	9	147	138	Few	Old	Few
15	30A	14	230	216	Many	Recent; old	Few
15	30B	14	230	216	Many	Recent; old	Few
16	31A	14	230	216	Many	Recent; old	Few
16	31B	14	230	216	Many	Recent; old	None
14	34B	12	232	220	Few	Recent	None
14	34B	12	232	220	Few	Recent; old	Very few
11	33	10	233	223	Many	Recent; old	Very few
9	32A	9	233	224	Few	Prominent	Many
9	32B	9	233	224	Few	Prominent	None
12	57	16	365	349	Few	Recent; old	Few
12	57B	16	365	349	Few	Old	Few
13	58A	16	365	349	Two	Old	Many
13	58B	16	365	349	Two	None	Few
8	56A	9	362	353	Many	Recent; old	Many
8	56B	9	362	353	One	Old	Many
6	55	8	362	354	Four	None	Many
				Guinea Pigs			
1	15	12	93	81	Two	Two old	None
4	23	10	143	133	Four	Old	Indication
4	24	10	143	133	Dozen	Old	None
3	21	14	159	145	Few	Few; old	None
3	22	14	159	145	Seven	Few; old	None
15	40A	6	200	194	Few	Old	None
15	40B	6	200	194	Few	Old	None
11	37A	16	223	197	Three	One; old	None
11	37B	16	223	197	Three	Old	None
12	38A	12	209	197	Four	Old	None
12	38B	12	209	197	One	Old	None
13	39A	8	205	107	Few	Old	None
13	39B	8	205	197	Few	Old	None
10	36A	90	303	213	One	None	None
10	36B	90	303	213	None	One; old	Few
5	35A	60	279	219	None	Old scar	None
5	35B	60	279	219	None	None	None
19	49A	6	317	311	Few	Old	None
19	49B	6	317	311	None	None	None
17	50A	8	319	311	Few	Old	None
17	50B	8	319	311	Few	Old	None
16	52A	6	317	311	One	Old scar	None
16	52B	6	317	311	Few	Old	None
20	53A	10	321	311	None	None	None

TABLE 1—*Continued*

Animal Number	Ovary Series	Age in Days		Days Post-Treatment	Developing Follicles	Corpora Lutea	Testis-like Tubules
		At Treatment	At Autopsy				
				Guinea Pigs—*Continued*			
20	53B	10	321	311	Nine	None	Few
14	54A	8	322	314	One	One; old	None
14	54B	8	322	314	Eleven	Few	None
9	51A	10	330	320	One	Old scars	None
9	51B	10	330	320	None	One; old	None
6	47A	8	343	335	Few	Old	None
6	47B	8	343	335	Few	Old	None
7	48A	8	343	335	Few	Old	None
7	48B	8	343	335	Few	Old	None
				Cats			
2	25	33	130	98	Numerous	None	None
2	26	33	130	98	Numerous	None	None
8	28A	46	190	144	Numerous	None	None
8	28B	46	190	144	Numerous	None	None
12	69	23	183	160	Numerous	None	None
11	70	23	204	181	Numerous	None	None
10	67	27	232	205	Numerous	None	None
9	68	27	244	217	Numerous	None	None
1	41A	33	257	224	Numerous	None	None
1	41B	33	257	224	Numerous	Large	??
3	44A	32	299	267	Many	None	None
3	44B	32	299	267	Many	None	None
6	59A	46	366	320	Numerous	Atypical	None
6	59B	46	366	320	Numerous	Large	None
				Opossums			
1	8	110	146	36	Numerous	None	None
1	12	110	151	41	Numerous	None	None
2	14A	110	169	59	Numerous	None	Good
2	14B	110	169	59	Numerous	None	Few
10	29A	78	214	136	Numerous	None	None
10	29B	78	214	136	Many	None	None
6	27A	69	220	151	Many	None	Solid
6	27B	69	220	151	Many	None	Solid
16	65	88	261	173	Numerous	None	None
11	43A	78	261	183	Numerous	None	None
11	43B	78	261	183	Numerous	None	None
19	71	119	339	220	Numerous	None	None
12	46A	78	318	240	Many	Large amt.	None
12	46B	78	318	240	Many	Large amt.	None
20	72	119	359	240	Numerous	None	None
7	42A	70	320	252	None	None	None
7	42B	70	320	252	Many	None	Good
17	66	88	339	251	Numerous	None	None
8	45A	70	379	309	Numerous	None	Good
8	45B	70	379	309	Numerous	None	None
15	73	87	428	341	Many	None	None

appearing ovocyte. Several corpora lutea were present, some of fairly recent formation and others showing large connective tissue-like centers with a rim of luteal cells. Medullary cords converted into testis-like tubules were scattered fairly well throughout the ovarian mass and displayed a branching condition, with individual areas of the tubules resembling cryptorchid testes; they represent a relatively small mass of tubules the central portion of which contains the characteristic Sertoli-like syncytium. A section through this ovarian mass is to be seen in Figure 1 and shows sections through adherent oviduct, a central cystic area, two large antrum-bearing follicles not entirely normal but each containing an ovocyte; the ovocyte of the upper follicle appears in another section. Two older corpora are present. Figure 5 is a higher magnification of some of the medullary tubules from the second ovary recovered from this animal. The second ovary was smaller than the first and contained one large antrum-bearing follicle, two lutein bodies, and small amounts of testis-like medullary cords (Fig. 5).

In this animal, therefore, the larger portion of both ovaries had been removed, and a total of 21 follicles of all stages in development were present. In each ovary, medullary components were present that appeared quite like degenerate testis tubules, but the quantity of such tubular mass was small and showed little tendency to undergo any pronounced development. Follicle activity was tremendously reduced, but follicles having a single layer of cells, those with a granulosum layer of 3–4 cells, and antrum-bearing follicles with good ovocytes were present 353 days after destruction of the germinal epithelium and the majority of the ovarian cortex.

Rat No. 9 (ov. ser. 32A and B).—Rat No. 9 was subjected to bilateral ovarian treatment on day 9, with autopsy on day 233, or 224 days subsequent to treatment. The rat had shown two cornified vaginal smears within the 8 days prior to autopsy.

Each recovered ovary was represented by a small amount of tissue, and neither ovary showed traces of germinal epithelium. A few follicles were present in each, but these were represented by all grades of activity from primordial follicles of a single layer of cells around the ovocyte to follicles with pronounced antra (Fig. 2). Corpora lutea were present, and in one ovary a fragmented ovocyte was noted within the corpus luteum, representing luteinization without ovulation.

One ovary contained clearly defined, modified medullary cords that appeared similar to degenerate testis tubules. Figure 6 is a portion of a section of this second ovary and illustrates the meandering nature and character of these modified medullary cords.

Rat No. 13 (ov. ser. 58A and B).—Rat No. 13 experienced treatment of both ovaries on day 16 and was autopsied on day 365, or 349 days after treatment. A cornified vaginal smear was noted within the 5 days prior to autopsy.

Each recovered ovarian mass was small and consisted of a relatively narrow rim of ovarian tissue about a central cystic cavity. One ovary contained a large degenerating follicle and a small follicle with solid granulosum about the normal-appearing ovocyte. The second one contained two large antrum-bearing follicles that were degenerating. In each recovered ovarian mass there were degenerating remains of old follicles, a considerable amount of rather dispersed modified medullary cords decidedly resembling degenerate seminiferous tu-

bules, and small remnants of corpus luteum tissue.

The two ovaries recovered from this animal showed a total of four follicles. How long these might have been retained beyond the day on which autopsy occurred is, of course, unknown. It is also unknown whether the approaching absence of follicles represents more than the almost complete destruction of ovarian tissue as a result of the treatment; in a few cases treated ovaries left no detectable remains.

In general, the 24 rat ovaries recovered at periods ranging from 54 to 354 days subsequent to treatment contained, without exception, from two to numerous follicles; corpora lutea were present in many cases. Modification of medullary cord tissue into testis-like tubules was present in 16 of the recovered ovaries. In 7 of the 24 treated organs there appeared to be small patches of surface epithelium in restricted areas that resembled germinal epithelium; and in one or two cases there was some evidence of activity; very occasionally, large cells, presumably destined to be future ovocytes, were visible in a small patch of epithelium, but more frequently such apparent germinal epithelium areas were not associated with underlying primordial follicles. In any case this epithelium would represent an extremely small fraction of a per cent of the original surface covering. Not more than 2 or 3 recovered ovaries were entirely devoid of some lutein-like body, and well-formed corpora were frequently present.

It becomes apparent from these considerations, therefore, that, although the cortical areas had been severely damaged by the treatments, no pronounced development of the medullary components had occurred. Ovaries approaching a total absence of follicles did not contain medullary cord hypertrophy with conversion to pronounced testis-like masses; and some ovaries containing numerous follicles showed the modification of medullary cords to as great an extent as those in which essentially all follicles had been eliminated.

Follicular activity in these ovaries subsequent to complete, and almost complete, germinal epithelium destruction had continued for periods up to 354 days. It becomes apparent, therefore, that the presence of such follicles, representing all stages in development from primordial ones up to antrum-bearing follicles and corpora lutea, raises the question of their origin. They could not have been formed by proliferation from germinal epithelium within the preceding 7–11 months in the 16 ovaries recovered the longest after treatment. The follicles seen to be actively developing in these recovered ovaries may have been present at the time of treatment and were sufficiently uninjured to be able to grow and develop during the subsequent period. On the other hand, one may consider the possibility of differentiation of the germ cells and follicles from cells deep in the cortex and perhaps undifferentiated at the time of treatment. In any event the notion that germinal epithelium is required for the proliferation of new germ cells and follicle cells at intervals characteristic of the oestrous cycle and that the life-span of any follicle is to be restricted to periods of a few days in length does not receive support from these experiments.

GUINEA PIGS

Seventeen guinea pigs were autopsied, and 33 treated ovaries were recovered and studied in serial section. Treatments were given at ages from postnatal days 6 to 90, and autopsy occurred on days 93–343, representing periods of from 81 to 335 days subsequent to treatment.

Guinea pig No. 13 (ov. ser. 39A and B).—Each ovary in this animal received one application of the collodion–salicylic acid solution on postnatal day 8, and autopsy occurred on day 205, or 197 days subsequent to treatment. The uterus at autopsy was essentially normal in general appearance.

Serial sections revealed that one ovary contained a total of 8–10 normal-appearing ovum-bearing follicles; these consisted of small follicles without antra and others with large antra. A few small patches of epithelium, possibly remnants of germinal epithelium that escaped destruction, were not associated with the development of primordial follicles. A few old corpora lutea were present, the better-preserved ones containing connective-tissue–like centers. The greater portion of most sections consisted of large and small cystlike cavities lined by a flat epithelium. Some of these cavities may represent old cystic follicles, but in other places the structure appeared to represent distended rete tissue; still others, lined by more columnar epithelium, may represent occasional epoöphoron tubules and possibly converted medullary cords. The latter, however, do not show any distinct resemblance to testis tubules.

The second ovary was quite similar in character and carried approximately a dozen follicles: some of these were small and solid, while others possessed a well-defined antrum. A few old corpora lutea were present. Many cystlike cavities, large and small, are conspicuous and possibly in some cases represent remains of degenerate cystic follicles, while others suggest expansions of cavities within the rete. No testis-like tubules were present, and no germinal epithelium remained.

Guinea pig No. 10 (ov. ser. 36A and B).—Guinea pig No. 10 was subjected to ovarian treatment on day 90 and was sacrificed on day 303, or 213 days subsequent to treatment. Forty-eight days after the ovaries were subjected to treatment, this female delivered a litter of healthy young—hence was pregnant at the time of treatment; in treating the ovary, the uterus was not seen, and the state of pregnancy was not then known. At autopsy the uterus was rated as normal.

Serial sections of the two ovaries recovered 7 months after treatment showed them to be essentially free from follicular activity. In one ovary a single follicle contained an ovocyte; interstitial tissue was prominent; and numerous large and small cysts were present throughout the ovarian mass. Some of these network areas are believed to represent rete tissue with distended spaces. In the second ovary no follicles were seen; one old corpus luteum, many large and small cystlike cavities, and well-preserved interstitial tissue made up the bulk of the recovered ovarian tissue. A small amount of medullary cord modification into testis-like tubules was present, the character of which may be seen in Figure 9.

Guinea pig No. 6 (ov. ser. 47A and B).—This animal is a representative of one of the longest periods of survival subsequent to ovarian treatment. Each ovary received the collodion–salicylic acid treatment on postnatal day 8, and the animal was autopsied on day 343, or 335 days after the treatment. Firm adhesions to fimbria, capsule, and oviduct had occurred, and the uterus was grossly normal. Both ovarian remnants were small.

One ovary was represented principally by degenerate follicular masses or interstitial tissue and a network of open, dilated spaces lined by a flat epithelium which presumably may represent rete tissue; a large fluid-filled space, or cyst, occupied the center of the gonad tissue.

Adhesions to the surface had totally eliminated the epithelial covering and the major portion of cortical tissue, but approximately a dozen follicles with contained ovocytes were present. These included medium-sized follicles with solid granulosum and a few follicles containing a definite antrum; but all antrum-containing follicles showed varying stages of degeneration; the egg was present in most of these, but it was usually fragmented or contained hyalinized cytoplasm. No large follicles were normal. Some fibrotic old corpora were evident, but none of recent formation was present. The second ovary was quite similar to the first but contained a smaller number of primordial follicles. A large cyst and numerous distended epithelial-lined spaces were present. All medium-sized or larger follicles showed degenerative changes, and no recent corpora were present. In neither of these recovered ovaries was there any evidence of germinal epithelium.

Guinea pig No. 20 (ov. ser. 53A and B).—Each ovary of this animal was treated on day 10 and was recovered on day 321, or 311 days subsequent to total germinal epithelium destruction and great cortical injury. Histological study showed one recovered ovary to be devoid of follicles and corpora lutea. The general stroma contained principally the multiple small cystic spaces which characterized the treated guinea-pig ovary in contrast to those recovered from the other species, as well as scattered interstitial tissue. The second ovary contained approximately 8–10 follicles in relatively good condition, represented by primordial follicles, 3–4-cell layered follicles, and antrum-bearing follicles. An entire cross-section of this ovary is reproduced in Figure 3.

Considering the entire group of guinea-pig ovaries, recovered at various periods after treatment, only a single one of the 33 was believed to contain any local remnant of the germinal epithelium, and this consisted of but a very small surface patch. Six ovaries among the 33 failed to show a follicle in some stage of development. Several of the ovaries contained from a single follicle to 3 or 4 follicles in a more or less normal-appearing condition. Some ovaries contained from 15 to 30 fairly normal-appearing follicles. Corpora lutea of some type—recent, old, degenerating—were present in all but 5 of the ovaries. Quite prominent in the recovered ovaries of this group were numerous, scattered, large and small cystlike spaces, some of which appear to represent rete tissue; these fluid-containing spaces were much more frequent in guinea-pig ovaries than in ovaries of the other three species of animals. Medullary cord development into testis-like tubules was found in only 3 of the recovered guinea-pig ovaries, despite the rather extreme reduction, or total absence, of follicular activity.

Relative to follicular activity, it is to be emphasized that in only 1 ovary was there any suspected germinal epithelium; yet follicular activity of some grade was present in all but 6. Most of the ovaries contained all grades of follicular development from primordial to antrum-bearing follicles; hence it becomes apparent that, up to 11 months after destruction of germinal epithelium, follicular activity is present. These facts require interpretation.

It is apparent that proliferation of the germinal epithelium at each oestrous cycle is not necessary in order for follicular activity to be prominent. Destruction of this peripheral layer is not followed by the disappearance of all follicles, within the period of an oestrous cycle or in the period covering many of them. If cells, formerly budded off from the germinal

epithelium and present as undifferentiated cells at days 6–8, could persist for long periods deep in the cortex and be able to differentiate periodically into young follicles capable of development to antrum stages, one could account for active follicles up to 11 months after treatment. We are not presenting any concrete evidence that such a condition accounts for our observations; but, unless some such continuous differentiation of residual cells is visualized, it would appear that one must attribute to a follicle a tremendously longer life-span than that of a red blood corpuscle.

CATS

Fourteen treated ovaries were recovered from 9 cats in which collodion–salicylic acid solution was applied to the ovaries on postnatal days 23–46, with autopsy on days 130–366, representing a post-treatment period of from 98 to 320 days' duration. In 4 animals 1 ovary had been removed at the time of treatment of the single remaining ovary. Consideration will be given to the ovaries recovered from 3 animals that represent the shorter and longer periods subsequent to treatment.

Cat No. 2 (ov. ser. 25 and 26).—Cat No. 2 was subjected to bilateral ovarian treatment on day 33, with sacrifice on day 130, or 98 days after treatment. At autopsy both ovarian remnants were small and firmly adherent to fimbria, oviduct, and upper uterus.

Serial sections of recovered organs presented essentially a picture of a normal ovary, with the exception that no germinal epithelium-covered surface existed; the ovarian tissue was firmly encapsulated by a connective-tissue mass in which oviduct was present. Follicles were numerous and were represented by large antrum-bearing follicles with normal-appearing ovocytes, smaller follicles of 2–3-celled granulosum, and typical primordial follicles with a single epithelial layer about the ovocyte. Many egg cells unaccompanied by surrounding follicle cells were scattered among connective tissue or ovarian stroma to constitute the so-called "naked ova." Several pluriovular follicles were present which contained two or three ovocytes that appeared normal. Corpora lutea were not seen. Some localized epithelial-lined spaces apparently represent rete tissue, but no testis-like tubules were seen. The characteristic interstitial tissue was conspicuous. No multiple large or smaller cysts, so apparent in the treated guinea-pig ovaries, were present, and for the most part the treated cat ovary was histologically quite normal in appearance. The second ovary was similar to the above and contained follicles of all grades of development, including pluriovular follicles.

Cat No. 6 (ov. ser. 59A and B).—Both ovaries of this animal were subjected to treatment on postnatal day 46, and the animal was autopsied on day 366, or 320 days subsequent to treatment. When the animal was approximately 9½ months old (on the 247th day following treatment), it was first observed to be in heat; it continued to exhibit oestrous behavior each time it was handled for the next 70 days until autopsy was performed. The condition of heat may have been present at an earlier period than it was observed, but a continuous state of oestrus existed for at least 70 days prior to autopsy. Each ovary was found to be firmly adherent to dorsal musculature, fimbria, oviduct, and upper uterus. The oviduct on one side was distended, and the uterus was somewhat enlarged and turgid.

Serial sections of the two ovaries showed them to be quite similar; each was much smaller than the normal ovary, but follicles were numerous, and all

stages of follicular development were represented. The majority of the large antrum-bearing follicles exhibited some grade of degeneration, and some approached a cystic condition. Pronounced follicular degeneration was evident in each ovary, but quantities of small follicles and follicles with small antra were normal in appearance. Many primordial follicles were present. An entire cross-section of one ovary (Fig. 12) readily demonstrates that numerous follicles are present and contain normal-appearing ovocytes. Under higher power the area outlined by the rectangle is reproduced in Figure 14 to demonstrate some of the numerous primordial follicles that are present in this ovary.

Two unusual and different types of structure were noted in each of the ovaries recovered from this animal. They were not seen in any other ovary. One type, relatively large and present in approximately one-half the sections, is apparently old and atypical corpora lutea. Four of these bodies were present in each ovary, and a section through one of them can be seen in Figure 13. The essentially solid structure is well circumscribed, contains sharply delimited, small, cleftlike spaces, and is made up of moderately sized, glandlike cells, resembling to some extent corpus luteum cells containing numerous vacuoles of varying sizes.

The second structure (4 in one ovary, 1 in the second ovary) was a meandering, lobulated, tubular structure, S-shaped or U-shaped, that contained a cavity frequently fairly well filled with a fibrous, stringy, or cornified-like mass of material that may represent a secretion or degeneration product. The folded, loosely arranged epithelial cells provide a wall of some 4–10 cells; peripherally, a distinct bounding basement membrane separates the structure from surrounding tissues. In Figure 12 sections are shown through two of these unnamed structures, and under higher magnification Figure 13 reveals one of these bodies in three sections; serial sections show them to connect at different levels, thus demonstrating their tortuous course in the substance of the ovary. Their exact nature or significance was not determined. Presumably, one or both structures contributed to the continuous oestrous state, and the conditions are, perhaps, not unlike the state of nymphomania in cows which is associated with a persistent cystic follicle.

Cat No. 3 (ov. ser. 44A and B).—Cat No. 3 was subjected to treatment of both ovaries 32 days after birth and was autopsied on day 299, or 267 days subsequent to treatment. Both recovered ovaries were relatively small and had formed extensive adhesions. Each contained quantities of developing follicles of all grades of development. No corpora lutea, testis-like medullary tubules, or germinal epithelium was present. An entire cross-section through one ovary (Fig. 15) shows the connective-tissue encapsulation of the ovarian remnant. Numerous follicles are present, including one with two ovocytes having the nucleus in the same plane of section.

A consideration of the entire group of treated cat ovaries suggests a few outstanding points: (1) None of the 14 treated ovaries, recovered 98–320 days after treatment at animal ages of 4 months to 1 year, was seen to have retained any trace of germinal epithelium. Cortical destruction, therefore, had been severe, and subsequent adhesions of the remaining tissues left no free epithelial-covered surface. (2) In only one of 14 recovered ovaries was there any tissue resembling degenerate testis tissue to suggest an activation of medullary components in the direction of testis-like

tubules. Animal No. 6 considered above, however, did show in each ovary peculiar unknown tubular structures somewhat resembling atypical degenerate tubules, but it appears to be a question whether these were of medullary derivation. (3) Each of the 14 recovered ovaries displayed quantities of active follicles, but follicular degeneration, especially of the larger follicles, was pronounced; many small as well as large follicles were undergoing degeneration. Quantities of multiovular follicles containing two or three normal-appearing ovocytes were present. (4) Corpora lutea were seen in the ovary of animal No. 6 and in one ovary of cat No. 1.

It is frequently difficult or impossible to judge whether a given follicle is a "medullary" follicle or one of the ordinary cortical follicles. Kingsbury (1913) has discussed the problem and in some cases is able to recognize the medullary follicle as a distinct type. In these recovered, treated ovaries, very abnormal relations are present. Many follicles are composed of a distinct single layer of cells surrounding the ovocyte, with nuclei located basally; a solid cytoplasmic area may be present between the follicle cells and the ovum, or there may be a space that suggests an antrum. Many of these, for various reasons, are believed to represent medullary follicles; and, if this is a correct interpretation, they have persisted over much longer periods than in normal ovaries, since Winiwarter and Sainmont (1908) claim their extinction by postnatal day 65.

The absence of testis-like tubules from converted medullary cords may possibly be accounted for by assuming an insufficient removal of cortex; presumably, one could expect medullary cord conversion toward testis-like structures only when cortical activity is reduced to a minimum, and in all recovered cat ovaries much follicular activity was apparent. On the other hand, it is possible that medullary cord activity follows a follicular type of activity and develops or becomes conspicuous only when germ cells are contained within the cord.

OPOSSUM

Twenty-one treated ovaries were recovered from 13 female opossums, of which one ovary was treated in 5 animals, the second one having been removed at the same time. Treatments were administered on postnatal days 70–119, with autopsies occurring on days 146–428, representing recoveries of 36–341 days following treatment. Since the first signs of antrum formation in the opossum occur about the 115th–125th postnatal day, all treatments were given prior to antrum formation.

Opossum No. 2 (ov. ser. 14A and B).—Two small ovarian remnants were recovered from this female at autopsy on day 169, with treatment applied on day 110, or a post-treatment period of 59 days. These two ovaries lacked any suggestion of a germinal epithelium, and the recovered ovarian tissue was surrounded and infiltrated by connective tissue, which graded into the fused oviducts and other peripheral tissue. Numerous follicles were present, and the smaller ones, especially, appeared entirely normal; many larger ones showed severe degeneration. A relatively abundant mass of medullary cord tissue was present, resembling to a considerable extent testicular tissue (see Fig. 8); it suggests an epithelial proliferation and formation of Sertoli-like lining; but most of the tissue does not appear to be healthy and active. In some regions the primary cords contain germ cells resembling ova; apparently, many medullary cords contain eggs and thus produce medullary follicles, but it is still difficult to be certain

in the identification of a medullary follicle. Some of these cords, containing ova, appear without question to connect with rete spaces. Many follicles consist of pyknotic or blackened and apparently dead cells. No corpus luteum tissue was present.

Opossum No. 1 (ov. ser. 8 and 12).—In opossum No. 1 both ovaries received treatment on day 110, and the animal was opened under anaesthesia on day 146, with removal of one ovary. On days 147, 148, 149, and 150, respectively, 12½ rat units of an equine gonadotropin (gonadin) were administered, and the animal was autopsied on day 151, at which time the second ovary was recovered.

The ovary removed at operation 36 days after original treatment (ov. ser. 8) contained many follicles, of which approximately one-half appeared entirely normal; others showed some degree of degeneration. Many follicles were primordial ones, others were small and solid, while many showed developing antra of a netlike character. No luteinization was present. The ovary removed after four daily gonadotropic treatments (ov. ser. 12) owed its larger size primarily to the tremendously increased follicular size. Numerous large follicles with large antra were intermingled with smaller solid follicles and follicles in which the antrum was just indicated. It was not certain which follicles, if any, were of medullary derivation. No corpus luteum was present. The gonadotropic effect in this case was apparently only follicle-stimulating.

Opossum No. 8 (ov. ser. 45A and B).—The ovaries of opossum No. 8 received the collodion–salicylic acid treatment on day 70, and the autopsy occurred on day 379, or 309 days subsequent to treatment; both ovaries were histologically quite similar. Each contained primordial, small solid, and small antrum-bearing follicles, along with many very large follicles, tending toward a cystic condition. Essentially all large antrum-bearing follicles showed some degenerative changes; some possessed but a thin stratum granulosum, and in some the ovocytes were fragmented, while still more degenerate conditions were represented by a fibrous or cornified type of degenerating body. No corpora lutea were present in either ovary. One ovary contained converted medullary cords resembling testis tubules, but this tissue was very small in amount. Some of these testis-like medullary cords are illustrated in Figure 11.

Opossum No. 12 (ov. ser. 46A and B).—Both ovaries received treatment on day 78, with autopsy occurring 240 days later (day 318). The animal is exceptional from the fact that it alone possessed corpora lutea; these were present in each ovary.

In Figure 4 an entire cross-section of one ovary reveals the presence of a large amount of corpus luteum tissue, but it also shows follicular activity of all grades from primordial follicles to antrum-bearing follicles. The enlarged marked square reproduced in Figure 7 permits the identification of three primordial follicles (see also Fig. 10). Modified medullary cords were not identified in either ovary.

A general consideration of the group of treated opossum ovaries revealed that, of the 21 recovered treated ovaries, only 3 showed a small patch or patches of suspected germinal epithelium and only 1 of the 3 provided suggestions of the formation of new germ cells. All recovered ovaries contained follicles representing all grades of differentiation from primordial to mature follicles; in general, the majority of the large antrum-bearing follicles were degenerate to some degree. Many pluriovular follicles were observed

(see Hartman, 1926). One animal sacrificed during the month of April yielded treated ovaries containing corpora lutea, but corpora were not present in 8 other ovaries recovered during February, March, and April, or the period of the year when ovulations normally occur in this strictly seasonal-breeding animal.

In terms of production of new ova and follicles from germinal epithelium, therefore, it can be emphasized again that quantities of follicles of all stages in development from inactive primordial to antrum-bearing were found 36–341 days after destruction of the germinal epithelium, as well as much of the underlying cortex, as a result of the surface application of the corrosive chemical. These follicles, therefore, have either persisted up to more than 1 year after formation, or they have arisen from the general substance of the ovarian tissue, possibly from otherwise undifferentiated residual cells and in the absence of any germinal epithelium. How much of the follicular activity is due to medullary follicles and how much to cortical follicles is difficult to determine.

DISCUSSION

The primary purpose in conducting this study on the ovary of rats, guinea pigs, cats, and opossums subsequent to the destruction of the germinal epithelium and various amounts of underlying cortex was an attempt to produce conditions that would favor the development of the medullary components of the ovary. It was thought possible that the cat and opossum ovary might prove exceptionally important in this attempt, since in both species it is usual to find a great deal of medullary cord activity in early life. This activity expresses itself usually in the formation of medullary follicles that are so characteristic of follicular development as to make it very difficult to determine whether a given follicle arises from primary sex cord activity or from the cortical cord components. It is believed that the follicular activity in medullary components is short lived and that all such follicles degenerate early and take no part in reproduction (Winiwarter and Sainmont, 1908; Kingsbury, 1913). Morgan (1943) noted them in adult ovaries in the opossum.

For years it has been considered that the medullary portion of the ovary was homologous to the testis and that its principal potential was in this direction. The most striking evidence for this conception of the mammalian ovary was provided by the study of the freemartin ovary, in which, owing to the unusual conditions prevailing, the cortical materials atrophied or were never developed, and the medullary components developed into a typical undescended testicle, including seminiferous tubules and rete apparatus; epididymis and the excurrent outlet as well as the masculine accessory organs of reproduction also were present (Willier, 1921; Bissonnett, 1924).

Lower vertebrates present striking evidence for the production of testes from ovaries as a result of various experimental procedures or from observations of naturally occurring phenomena. Thus, in fish, Essenberg (1923) produced evidence that in *Xiphophorus* females egg production gradually ceased with age, cortical areas underwent degeneration, medullary portions hitherto relatively inactive assumed activity, and the ovary was gradually changed to a testis, thus converting the former female into a functional male. In Amphibia, Witschi (1929, 1939) demonstrated that, from developing eggs of the grassfrog maintained in water 10° above normal, only male development occurred; presumably, the conditions either inhibited cor-

tical development in female-determined embryos or medullary cord development was decidedly favored. In a general sense the medulla is regarded as testicular in potency, whereas the cortex is strictly ovarian. Humphrey (1942) was able to modify the development of an ovary of *Amblystoma* by transplantation of an embryonic testis; cortical development was in a less favored position, hence was removed, while the medulla was uninhibited and produced a testis. Breeding experiments proved that the medullary portion of the ovary produced spermatozoa that were fertile. The medullary portion of the right ovary in birds similarly revealed its capacity for development into testicular structures in the investigations of Benoit (1923) and Domm (1929). Brode (1928) revealed that the right rudimentary ovary, composed usually of only primary sex cords, contained primordial germ cells in the early stages and that the majority of these cells were gone soon after hatching. In Domm's experiments (1927, 1929) removal of the left functional ovary was followed almost invariably by growth of the right rudimentary structure, but the growth and development were usually testicular in character. If such removal occurred prior to loss of primordial germ cells, the testis frequently developed to the point that spermatogenesis resulted in the formation of motile spermatozoa.

Thus in many forms lower than mammals the testicular potency of the ovarian medulla has been amply demonstrated. In mammalian forms, however, the freemartin, with perhaps additional cases in twin heterosexual pig development, appears to be the best expression of the potencies of medullary cords to produce a testis. Naturally occurring ovotestis and the neoplastic arrhenoblastomatas of women, with their masculinizing effects, are other evidences of the potency of the medulla to express its inherent male potencies. Thus far, application of experimental procedures has not been generally successful in demonstrating these masculine potencies in the mammalian ovary, and it was hoped that our experiments might contribute some evidence that would lead to further clarification of the problem involved.

In this series of experiments, however, our procedures have not given the anticipated results. Despite the elimination of the germinal epithelium and variable quantities of underlying cortical tissue, in some cases with almost complete elimination of follicular activity, we have seen relatively little evidence for the conversion of medullary cords into testis-like material. In the rat ovary, converted medullary tubules most nearly approached a testicular development, but the degree of such development and the total tissues involved were but little, if any, better than that formerly seen by Buyse (1935) and Moore and Price (1942) in recovered ovaries subjected to transplantation during the embryonic stage. Some tendency was also seen for such development in the treated guinea-pig ovary, but less modification was noted in cat and opossum ovaries.

In mice the observations of Brambell, Parkes, and Fielding (1927), on young ovaries subjected to X-ray treatment during early stages, revealed total loss of ovocytes and follicles and the formation of tubular structures from subsequent proliferation of the germinal epithelium. These were considered to be somewhat like medullary cord tubules but represented, apparently, an abortive type of follicular development or "anovular follicles" (Brambell and Parkes, 1927).

The possibility remains, therefore, that, in order to realize more fully the developmental potency of medullary cords in mammals to produce testis

tubules, modifying influences will have to be applied at very early stages in ovarian development. Androgen treatment of female opossums during such early stages, however, failed to bring out any such tendency.

The second ovarian problem to receive our attention was not a primary concern in the initiation of this study, but it is one that deserves some consideration in the light of our observations; this is the problem of the origin and development of the germ cells and follicles.

The older notion concerning the origin of the female germ cells prevailed from the early study of the ovary by Pflüger in 1863 up to approximately the first quarter of the present century; and, indeed, it has prevailed to some extent up to the present time. It has been assumed that the germinal line was to a large extent separate from the soma; that the germinal cells were formed very early in development and set aside for the specific purpose of producing the definitive germ cells of reproductive life; and that all ova ever to appear in the ovary were produced during embryonic life.

Some slight modification of the prevailing notion was introduced by Winiwarter and Sainmont (1908) in their monographic treatment of development in the cat ovary. While recognizing the formation of germ cells and follicles during embryonic life, these investigators believed degeneration accounted for all the early differentiated germ cells and that succeeding follicles and ovocytes were differentiated from the germinal epithelium up to the period of sexual maturity; at this period all definitive ova of reproductive life were present. A somewhat similar contention was advanced by Kingery (1917), who recognized two periods of proliferation of germinal cells. The first proliferation period occurred before birth and produced the primitive germ cells, all of which were overtaken by degeneration. The second period occurred between birth and sexual maturity to provide the definitive germ cells of reproductive life. Rather than existing as specialized cells, however, Kingery believed that "apparently any cell in the germinal epithelium is a potential egg cell" (p. 277). Robinson (1918), however, advanced the notion that in the ferret new ova and follicles cells were formed throughout the whole period of functional ovarian life, and Arai (1920) claimed that in the rat ovogenesis continued up to 1 year.

The newly emerging idea that germ-cell formation occurred in the ovary throughout reproductive life was given decided emphasis by Allen (1923). In a careful study of ovaries of mice, closely timed in relation to the oestrous cycle, Allen presented strong evidence on the point that ovogenesis occurred cyclically throughout reproductive life, with accelerated formation of new germ cells from the germinal epithelium at the time of oestrus. As new germ-cell differentiation occurred, the older germ cells and follicles were overtaken by degeneration; hence the life of a given germ cell or follicle was quite limited. Enumeration of new germ cells in several ovaries suggested that from 400 to 500 new ova were differentiated in one ovary during each normal oestrous period, with an average of 4½ ova reaching maturity. This represented approximately a 1 per cent survival of ova formed at each wave of differentiation. Hargitt (1930*a*) is in essential agreement that cells arising from germinal epithelium activity become transformed into definitive ova: "This process of transformation seems to be a continuous one, and no ovum or follicle can remain long in a dormant condition —it must either grow and mature or else degenerate" (p. 317). As indicated in the

introduction, the extreme of this point of view is represented perhaps by the publication of Evans and Swezy (1931), in which support is given to the idea that in mammals in general the life of a germ cell or follicle is less than the period of one oestrous cycle.

This now firmly established notion that ovogenesis is a process occurring throughout reproductive life in the ovary has not received unanimous concurrence. Among others who failed to find evidence to support the conception or who studied the process only prior to sexual maturity may be mentioned Winiwarter and Sainmont (1908), Kingery (1917), Cowperthwaite (1925), Pincus and Enzyman (1937), Kingsbury (1938), Sneider (1940), Nelsen and Swain (1942), Everett (1943), Bookhout (1945). The theory that germ-cell differentiation occurs in the ovary during sexual maturity and that the life of a follicle is very short has been supported by Robinson (1918), Arai (1920), Allen (1923), Butcher (1927), Hargitt (1930*a* and *b*), Evans and Swezy (1931), Smelser, Walton, and Whetham (1934), Guthrie and Jeffers (1938), Duke (1941), Morgan (1943), Latta and Pederson (1944), and Barton (1945).

In the majority of studies directed toward the solution of the problem of the origin of germ cells in the ovary, reliance has been placed upon somewhat incidental observations or upon the study of a closely graded series of ovaries from normal females. Some experimental procedures, however, have been applied to the study. Brambell, Parkes, and Fielding (1927) and Brambell and Parkes (1927) subjected the ovaries of mice to X-ray treatment prenatally, shortly after birth, and in adult life. Complete destruction of ovocytes and follicles could be obtained. Later proliferation from the germinal epithelium in mice treated shortly after birth produced cords of cells growing into the ovarian substance. These produced tubular-like masses of cells. Although the activity of the germinal epithelium produced ingrowths of cords of cells, no germ-cell differentiation was noted.

Everett (1943) utilized radiation on the ovaries of mice, as well as the transplantation of young whole ovaries to a host kidney, or the genital ridge of embryos prior to entrance of primordial germ cells from the gut. His conclusions from the study embody the notion that the cells of the germinal epithelium, which form ova during postembryonic life, are derived from the primordial germ cells. Latta and Pederson (1944) attempted the use of India ink injections into the periovarial space of mice in order to trace carbon particles taken up by cells in the germinal epithelium; they later identified ink particles in germ cells and in follicle cells located considerably below the surface layer.

The studies on series of normal ovaries, as well as the conclusions derived from experimental studies on the problem, appear to establish without doubt that new ovocytes and follicle cells can differentiate in the ovaries of mammals after the period of attainment of sexual maturity and perhaps throughout effective reproductive life. They likewise suggest, but do not prove, that the total life-span of an individual follicle and contained germ cell is of very short duration. The suggestion that many follicles are overtaken, and effectively removed, by degeneration does not constitute proof that all follicles and germ cells suffer the same fate. It must also be admitted, as a possibility, that undifferentiated cells within the ovarian substance may, under proper conditions, undergo differentiation into germ cells and follicle cells. It is believed that the observations

presented in the body of this paper and obtained from a study of the effects following the elimination of the germinal epithelium of the young ovary preceding sexual maturity, by means of the salicylic acid treatment, will force a reconsideration of this entire process.

The bare facts to be considered are that, subsequent to destruction of the germinal epithelium as well as of various depths of cortical tissues by means of corrosive chemical substance applied to the entire surface of the very young ovary, the recovered treated ovary continues to reveal follicles in active growth and development for long periods of time. We have noted follicles in all stages of development from inactive primordial follicles to those with small antra and larger antrum-bearing follicles undergoing degenerative changes; in many recovered ovaries corpora lutea of various grades are seen, some of which inclose visible ococytes undergoing fragmentation. This condition has been seen to prevail in the treated rat ovary for periods up to 354 days subsequent to germinal epithelium destruction; in the treated guinea-pig ovary up to 335 days; in the treated cat ovary up to 320 days; and in the opossum ovary up to 341 days. Similar conditions prevail in those ovaries removed as early as 54 days after treatment in the rat, 81 days in the guinea pig, 98 days in the cat, and 36 days in the opossum. Such follicular activity, ever present over a range of practically a year subsequent to treatment in each species, suggests that the process of follicular development has been a continuous one. In 6 recovered guinea-pig ovaries and 1 cat ovary no follicles containing ovocytes were noted. The recovered cat ovary proved to have very little recognizable ovarian tissue remaining, and the mass consisted largely of oviduct. Some of the guinea-pig ovaries did contain appreciable amounts of ovarian stroma, remnants of old corpora lutea, degenerate follicular masses, and many cystic structures, some of which are probably of follicular origin. It is unknown whether the absence of follicles containing ovocytes represents a more sensitive response on the part of the guinea-pig ovary or whether in these cases more cortical tissue was killed at the time of treatment. It is possible that a few follicles may have escaped destruction at the time of treatment and, in the subsequent 7–10 months, may have been removed through their development up to the point where, in the absence of ovulation, degeneration would be expected to follow.

It appears that these observed facts warrant the interpretation that continuous follicular activity in mammalian ovaries, for a period of at least a year, does not depend upon an intact and active germinal epithelium to provide new germ cells and follicles. If this is justifiable, it would seem that one of two general conditions must prevail in ovarian follicular activity: (1) either germ cells and follicles, present deep in the ovary at the time of treatment, can remain in the ovary and undergo maturity phenomena at any time up to a year, or (2) undifferentiated germ cells and potential follicle cells, present at the time of treatment, can differentiate at any time up to a year, thus keeping the ovary supplied with active follicles containing germ cells. If the first possibility proves to be the correct interpretation, the conclusion would have to follow that the life-span of a formed follicle is tremendously greater than recently developed conceptions would allow. If the second possibility proves to be the correct one, then we should of necessity deny the contention that all germ cells and follicles present during reproductive life arise directly

from the germinal epithelium. The possibility must be entertained that germ cells and follicles may arise both from the immediate activity of the germinal epithelium and from later differentiation of previously undifferentiated cells located within the substance of the ovary.

Our materials, as in the case of former investigations, fail to provide a convincing answer to the problem. Some procedure by which individual follicles can be clearly marked and their subsequent fate followed without question will be required to give a convincing answer to these now unsettled problems.

SUMMARY AND CONCLUSIONS

1. Mammalian ovaries from four species were completely covered with a corrosive chemical substance—salicylic acid dissolved in collodion—prior to follicular maturity, in an attempt to destroy the young germinal epithelium and various depths of underlying cortex.

2. A total of 92 recovered ovaries has been studied in serial section. These consist of 24 rat ovaries, treated as early as postnatal day 8 and recovered at autopsy as late as 365 days subsequent to treatment; 35 guinea-pig ovaries, treated as early as day 6 and recovered up to 343 days after treatment; 14 cat ovaries, treated as early as day 23 and recovered up to 320 days later; and 21 opossum ovaries, treated as early as day 69 with recovery up to 325 days after treatment.

3. Despite complete destruction of the young germinal epithelium, and in some ovaries sufficient cortex to remove follicular activity almost completely, very little medullary cord development in the testicular direction occurred.

4. Prepuberal destruction of germinal epithelium and underlying cortex permits the continuance of active follicular development up to at least 1 year later in the majority of cases. Primordial, to antrum-bearing, and larger degenerating, follicles are present.

5. The presence of an active peripheral germinal epithelium during reproductive life is not a necessary requirement for the presence of active follicular development for a period of at least a year.

6. It would appear that (*a*) differentiated germ cells and follicles can persist within the ovary for a year in small animals having a total reproductive life of but a few years or (*b*) that undifferentiated ovarian cells are capable of developing into germ cells and follicles.

7. The problems of germ-cell origin and follicular life in the mammalian ovary are still problems awaiting solution.

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PLATE I

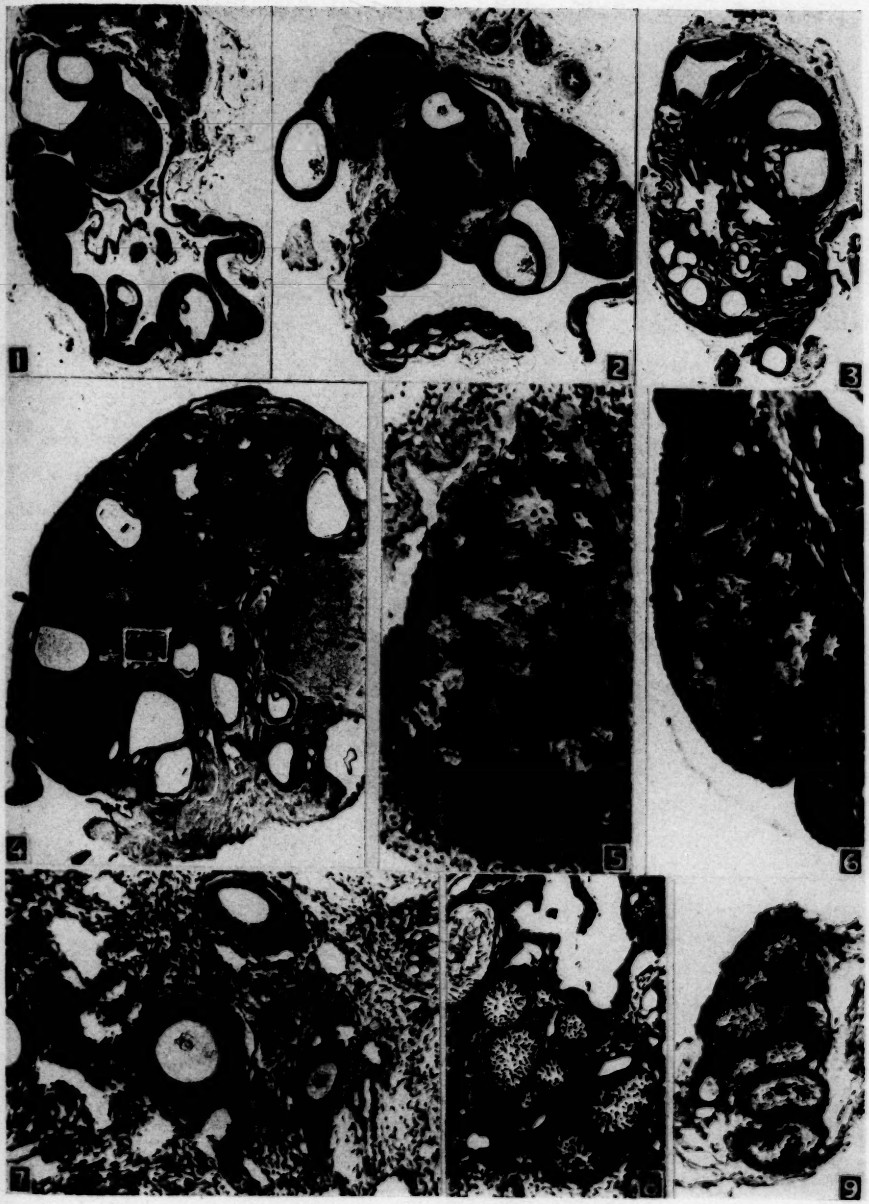

PLATE II

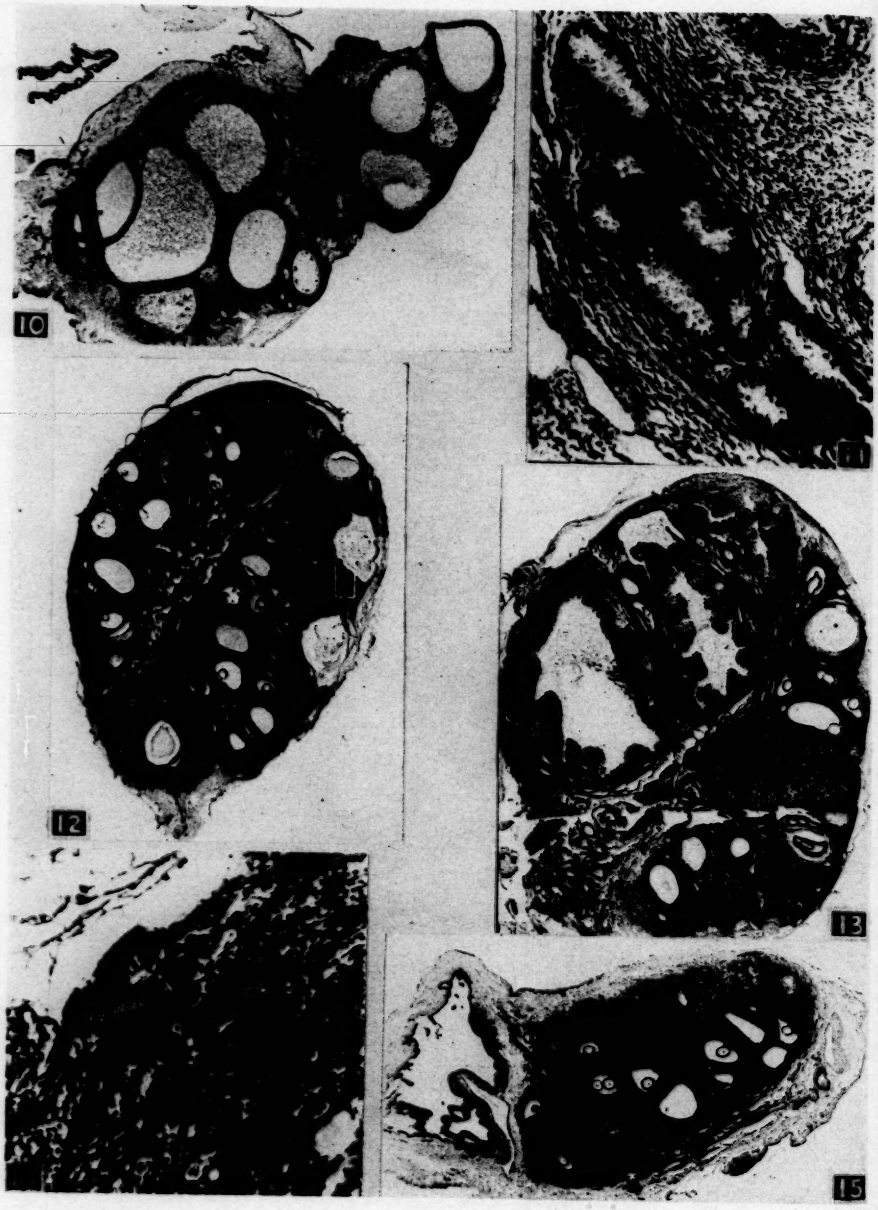

PLATE I

FIG. 1.—Rat No. 8 (56A). Entire section through ovary, 353 days post-treatment (one treatment each ovary, day 9; autopsy, day 362). Note oviduct above, cystic central cavity, two antrum-bearing follicles, above and below (ovocyte present), two corpora lutea. ×20.

FIG. 2.—Rat No. 9 (32B). Entire section through ovary, 224 days post-treatment (one treatment, each ovary, day 9; autopsy, day 233). Note three antrum-bearing follicles, ovocytes appearing in other sections; one small follicle next to large follicle above, several corpora lutea; adherent oviduct above and right. ×20.

FIG. 3.—Guinea pig No. 20 (53B). Entire section through ovary, 311 days post-treatment (one treatment, each ovary, day 10; autopsy, day 321). Four antrum-bearing follicles, ovocyte showing in large central follicle, and indistinct small follicles. Note fluid-filled spaces throughout section. This ovary did not contain distinct corpora lutea. ×20.

FIG. 4.—Opossum No. 12 (46B). Section through ovary, 240 days post-treatment (one treatment, each ovary, day 78; autopsy, day 318). Note antrum-bearing follicles and corpora lutea. Area marked in square more highly magnified in Fig. 7. ×15.

FIG. 5.—Rat No. 8 (56B). Section shows medullary cords modified into tubules resembling degenerate seminiferous tubules; see caption for Fig. 1 for history. ×170.

FIG. 6.—Rat No. 9 (32A). Section shows medullary cords modified into tortuous tubules resembling seminiferous tubules. See caption for Fig. 2. ×170.

FIG. 7.—Opossum No. 12 (46B). More highly magnified area marked in Fig. 4. Note four nonantrum follicles and three primordial follicles, center, below. See caption for Fig. 4. ×130.

FIG. 8.—Opossum No. 2 (14A). Portion of section, 59 days post-treatment (one treatment each ovary, day 110; autopsy, day 169). Medullary cord structures frequently termed "anovular follicles"; distended rete above. ×130.

FIG. 9.—Guinea pig No. 10 (36B). Modified medullary cords resembling degenerate seminiferous tubules from ovary, 213 days after treatment (one treatment each ovary, day 90; autopsy, day 303. Animal pregnant, delivered normal young, 48 days post-treatment). ×170.

PLATE II

FIG. 10.—Opossum No. 19 (71). Entire section through ovary, 220 days post-treatment (one ovary removed, day 119; second ovary one treatment; autopsy, day 339). Note small follicles, antrum-bearing follicles, and cystic follicle; no corpora lutea. ×20.

FIG. 11.—Opossum No. 8 (45A). Portion of section through ovary, 309 days post-treatment (one treatment each ovary, day 70; autopsy, day 379). Medullary cords resemble degenerate testis tubules. Rete barely indicated at lower right corner. ×130.

FIG. 12.—Cat No. 6 (59A). Entire section through ovary, 320 days post-treatment (one treatment each ovary, day 46; autopsy, day 366). Numerous antrum-bearing follicles; corpus luteum above; peculiar body above and right (see Fig. 13). Area outlined is more highly magnified in Fig. 14 to show presence of primordial follicles. ×15.

FIG. 13.—Cat No. 6 (59A). Section from same ovary as Fig. 12. This figure is approximately 175 sections from Fig. 12 and shows three sections (above and left) of the peculiar structure present. Corpus luteum, center and right. ×20.

FIG. 14.—Cat No. 6 (59 A). More highly magnified area indicated in rectangle on Fig. 12, to show presence of primordial follicles (320 days post-treatment). ×200.

FIG. 15.—Cat No. 3 (44B). Entire section through ovary 267 days post-treatment (one treatment each ovary, day 32; autopsy, day 299). Antrum-bearing follicles, and one follicle showing two eggs with nuclei in same plane of section. ×20.

NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

ACTIONS OF RADIATIONS ON LIVING CELLS[1] BY D. E. LEA

Reviewed by RAYMOND E. ZIRKLE
University of Chicago

THE author of this book, as he points out in the Preface, makes no pretense of covering the entire field of radiobiology. On the contrary, he has elected to discuss in considerable detail certain radiobiological actions which are fairly amenable to quantitative treatment, namely, the inactivation of viruses, the production of gene mutations, and the induction of structural changes in chromosomes. There are also short treatments of delay in cell division and of certain lethal effects. Other aspects of cellular radiobiology and all aspects of the radiobiology of complex organisms are omitted.

Chapter i (32 pp.) is a short, but very adequate, treatment of the physical properties and methods of measurement of the various types of radiation. This chapter is deliberately and wisely written at a rather elementary level.

Chapter ii (36 pp.) is a discussion of the chemical effects of ionizing radiations and the possible relationships between chemical and biological effects. Emphasis is placed upon chemical reactions in aqueous solutions, and considerable attention is paid to indirect action of radiations upon solutes in aqueous solution.

Chapter iii (31 pp.) is a rather extended exposition of the target theory of radiobiological action. The concepts introduced here are extensively used in the remaining chapters.

Chapter iv (26 pp.) is a discussion of inactivation of viruses by various radiations. Experimental techniques are described, and direct and indirect modes of action of radiation are discussed. Much attention is paid to various types of evidence that the inactivation of a virus particle is due to a single ionization.

Chapter v (62 pp.) is a rather detailed treatment of the production of mutations of various sorts. Considerable space is devoted to descriptions of genetic changes and the techniques for detecting them. There are extended discussions of the effects of dose rate and type of radiation upon the mutation rate, of the relation between lethal mutations and structural changes in the chromosomes, and of the various deductions, which, from the quantitative radiobiological data, can be made concerning the size of the gene.

Chapters vi and vii (56 and 37 pp., respectively) deal with the production of structural changes in chromosomes and parts of chromosomes. The various types of induced structural changes are described at length. The various factors upon which depends the number of changes per unit dose are fully discussed. Chief of these are the dose rate, the total

[1] Cambridge: At the University Press; New York: Macmillan Co., 1947. Pp. 402. $4.50.

dose, and the density of ions produced in the tracks of the ionizing particles.

Chapter viii (25 pp.) is a discussion of the delay in cell division sometimes observed after irradiation; and chapter ix (37 pp.) deals with certain comparatively simple lethal effects, with special reference to relations between lethal action and genetic effects of various sorts.

The Bibliography is short but well selected. It suffers somewhat from the fact that the author has chosen to paraphrase many of the titles rather than to cite them as they appear in the original publications. The book is well indexed and contains many charts and four plates. The plates suffer from inadequate reproduction.

On the whole, the reviewer recommends this book as an excellent presentation of those fields of radiobiology which the author has selected for treatment.

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